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Introduction

FOREST nursery practice has for its main objective the production of the best stock possible for reforestation. Nurserymen all over the country have come to realize the importance of soil fertility in the production of planting stock. There is abundant literature on the nutrition of agricultural crops but only in recent years have the nutritional needs of forest trees received much consideration. Before a complete understanding of the application of fertilizer to the forest nursery is attained, we must first determine the fundamental relationships between the nutrients supplied and the seedlings to be grown. Nutrient requirements of species differ (Mitchell and Chandler, *21)*, and the demands upon the soil for each species must be determined to assure successful nursery production. After we have ascertained the nutritional requirements of a given species, we must then determine a practical method of applying the fertilizer in the nursery and at the same time provide optimum growing conditions.

Jack pine *(Pinus banksiana* Lamb.) was chosen for this study because it responds well to nutrients and because it is a forest tree becoming increasingly important in the Lake States. Since the sandy jack pine soils of this region are usually deficient in nitrogen, the effect of that element upon the growth and drouth resistance of jack pine seedlings was studied in detail. In the areas where the drouth resistance of a species determines the success or failure of a plantation, it is very important that we produce not only large seedlings by fertility treatments but drouth-resistant ones as well.

Three sand culture experiments to determine the effect of varying concentrations of nitrogen on the growth and drouth resistance of jack pine seedlings were made in the forestry greenhouse, University Farm, St. Paul, Minnesota. The work was started in the winter of 1938-39 and completed during the winter of 1940-41. In addition, a pot culture test of Cloquet nursery soil and a nursery field experiment were conducted during the summers of 1939 and 1940 in the Cloquet Forest Nursery, Cloquet, Minnesota. Each experiment will be discussed separately.

Review of Literature

The problem of nitrogen and plant growth has been studied by many investigators, but relatively few references are available on nitrogen in the growth of tree seedlings. Mitchell *(18, 19, 20,* and *21)* has published more on the effect of nitrogen on seedling growth than any other worker. He grew Scotch and white pine seedlings in sand cultures, studying the effect on growth of various concentrations of nitrogen as well as other elements. Growing about 25 seedlings in each jar, he found that growth was best when they received 300 p.p.m. of nitrogen. Beyond this point seedling weight decreased. The root/shoot ratio, on the other hand, decreased with increase in nitrogen, the largest ratio being below the optimum nitrogen concentration for growth alone. The percentage of nitrogen in the seedlings rose with increase in nitrogen supply. This relationship was almost rectilinear up to 300 p.p.m. At the optimum level of nitrogen the seedlings contained 3.26 per cent of that element. Chapman *(6)* reports this same relationship between external and internal nitrogen in yellow poplar seedlings. Correlating the nitrogen content of jack pine needles with the spacing in a thinning experiment, Adams *(1)* found that the percentage rises with increased spacing. This seems logical since the trees farthest apart would have a greater supply of nutrients available. Increase in nitrogen content with increase in supply is not peculiar to tree seedlings alone but has been found to occur in many plants, by Beckenboch, Robbins, and Shive *(4)*, Hoagland *(13)*, Sessions and Shive *(23)*, and Das *(8)*.

Nitrogen stimulates seed production as well as the growth of tree seedlings. Chandler *(5)* found that the number of seeds produced was greatly increased by application of nitrogen. This was true for several deciduous trees, particularly sugar maple and beech.

Several investigators have studied the relation of nitrogen to drouth resistance. Many diverse results have been obtained, but only those that are concerned with pine seedlings will be mentioned here. Shirley and Meuli *(25)* grew jack pine seedlings in three levels of nitrogen and found that with an increase in the fertilizer there was a decrease in drouth resistance. They tested drouth resistance in a machine especially constructed for the purpose (Shirley, *24*). Quite different results were obtained by Wilde (*30*). He grew jack pine seedlings in nursery beds supplied with different concentrations of fertilizer, and then planted them out in the field. At the end of three years he correlated the survival with the amount of fertilizer the seedlings had received while they were in the nursery. The survival was just as good for seedlings that had received nitrogen as for the ones grown in a nursery soil definitely deficient in that element. Wilde points out the importance of a well-balanced nutrient supply in producing good planting stock, and suggests that it is lack of nutrient balance that decreases drouth resistance and not just application of nitrogen. A balanced nutrient supply is not only essential to drouth resistance but it is a prerequisite to the production of any vigorous growing stock (Herbert, *12*, and Wilde, *29*).

The use of nitrogen fertilizer in the nursery has spread rapidly in recent years. Wahlenberg (*27*) found that if Engelmann spruce is properly fertilized, one year can be saved in the production of five- and six-year-old planting stock. For species grown two or three years in the nursery, not much time can be saved but much larger planting stock can be produced by proper use of fertilizer (Lunt, *16*, and Larsen and Stump, *15*). Wilde (*28*) has developed a method of applying fertilizer to seed beds that has considerable promise. He fertilized the beds with a suspension made of duff, ammonium sulfate, ammonium phosphate, and potassium nitrate. The humus acts as a buffer and also absorbs some of the nutrients. These absorbed nutrients are released in the soil slowly, thus making nutrients available for a longer period of time.

Nitrogen fertilizer can also be applied to soil by dissolving it in irrigation waters. Babcock (*3*) describes a very successful method of fertilizing orchard trees with readily available nitrogen. The nitrogen in a dissolved state was carried through the ditches with the irrigation water. This is an excellent way of adding nitrogen often in a form usable at once by the trees.

Nitrogen and Seedling Growth

In 1939 a greenhouse sand culture experiment was started to determine the optimum concentration of nitrogen and the effect of variation from that concentration on the growth of jack pine seedlings for the first growing season. The work was started on February 4 and completed April 28, giving the seedlings 83 days to grow in the culture solutions. The seedlings, however, had been germinated in sterile sand and were several days old before they were transplanted to the sand culture setup.

EXPERIMENTAL METHOD

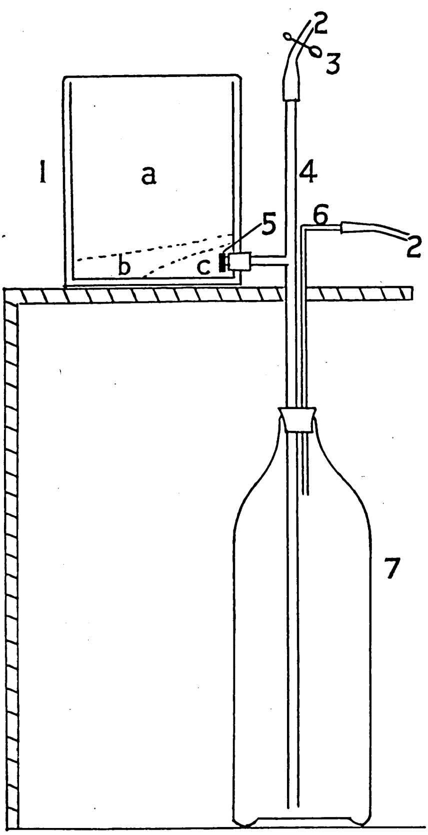


FIG. 1. Diagram of the sand culture apparatus

1. One-gallon glazed earthenware jar containing: (A) coarse sand, (B) coarse gravel, and (C) very coarse gravel; 2. rubber tubes on the end of the glass tubes; 3. clamp; 4. glass supply tube for transfer of solution from supply bottle to jar and back; 5. monel metal screen to prevent sand from getting into supply bottle; 6. glass air tube (air pumped into the bottle through the tube will force the solution in the supply bottle up into the jar of sand—with release of pressure the solution drains back into supply bottle); 7. supply bottle.

Sand culture apparatus. Following Eaton's *(10)* idea, Mitchell *(18)* developed a sand culture apparatus that worked very well for his experiments. The apparatus used in this experiment was, with the exception of a few modifications, very similar to that used by Mitchell. A diagram of an individual setup is shown in figure 1. One-gallon glazed earthenware jars were used to hold the sand. The culture solution was placed in one-gallon supply bottles, and by pumping air into each of the bottles through the glass tube at the top of the bottle, the solution was made to rise through the sand. This system of irrigation from the bottom helps to assure proper aeration of the roots growing in the jar, for when the pressure is released in the supply bottle the solution drains back into the bottle drawing a new supply of air into the sand. To insure proper drainage it was necessary to place some very coarse gravel over the opening in the lower part of the jar. On this was placed a layer of coarse gravel and then the jar was filled with sand (see figure 1). After all of the jars were filled with dry sand, 3½ liters of culture solution was placed in each of the supply bottles. The jars were then flushed and allowed to drain. The sand held a certain amount of the original solution but there was the same total volume present in each system (jar of sand and supply bottle). Next a mark was placed at the upper level of the solution in each supply bottle. In order to maintain the same volume of solution in the apparatus, the supply bottles were filled up with distilled water to that mark each day before the jars were flushed. This system allows for greater accuracy in controlling the amount of solution used and the concentration of the nutrient elements in it.

Sand. A rather coarse sand was necessary to make the apparatus work properly. Sand of the proper texture was obtained from a construction company in Duluth, Minnesota. This sand had been dredged from the bottom of Lake Superior a mile and one half from shore and by analysis contained 0.0015 per cent nitrogen. This amount is below the experimental error and for all practical purposes may be considered zero. The sand was washed with hot water to remove soluble nutrients present and colloidal material.

Culture solution. Since the solution that Mitchell *(18)* used had proven very satisfactory for white and Scotch pine, it seemed logical that it might also work for jack pine. Normally jack pine grows on a much poorer soil than white pine and for that reason its nutrient requirements may be somewhat different. As a test, an experiment was set up using varying concentrations of Mitchell's complete solution. It was found that about one-half strength gave satisfactory growth and this concentration was used for the first sand culture experiment.

There were 10 levels of nitrogen and 2 replications, making a total of 20 jars in this first experiment. A standard culture solution containing all of the essential elements, with the exception of nitrogen, was prepared. Three and one-half liters of this solution was placed in each of the 20 supply bottles. The concentration of that solution is given in table 1.

Table 1. Standard Culture Solution—Sand Culture Experiment, February to April, 1939

Source	P.p.m. of source	Mg. for 3½ L.	Concentration of nutrient element, p.p.m.					
			P	K	Ca	S	Fe	Mg
KH_2PO_4	556.0	1,946.0	126.7	159.7	......	......	......	......
$MgSO_4.7H_2O$	890.0	3,115.0	......	......	......	115.8	......	87.8
$CaCl_2.2H_2O$	667.5	2,336.2	......	......	181.9	......	......	......
Iron citrate	10.0	35.0	......	......	......	......	1.7	......

Unfortunately, during the last part of March, algae began to grow in the supply bottles because the bottles had not been properly protected from light. Figure 2 shows the general arrangement of the sand culture apparatus. At first it was thought that covering the front of the boxes with canvas, seen rolled up at the base of the jars, would be protection enough against the entrance of light. After this was found unsatisfactory, the top of each supply bottle was wrapped in tar paper and then a large piece was placed around all of the bottles. In addition to this, the canvas was tacked down over the open face of the box holding the earthenware jars. No more trouble with algae was encountered after the algae-infected solution had been replaced on March 27 by clean solution of the same concentration as the original. At the beginning of the experiment 3½ liters of standard solution had been placed in each supply bottle, and when the jars were flushed and allowed to drain, marks were placed at the upper limits of the solution in each of the bottles. It was found that there were 2.1 ± 0.1 liters left in each of the bottles. When the fresh solution was placed in the bottles, 2.1 liters was placed in each.

On April 18 an addition was made to the standard solutions in the supply bottles. A concentrated solution was prepared and placed in each bottle in the proper amount to increase the concentration by one third the original value. The values of this addition can be represented by one third the values given in table 1. This addition and the replacement described above were the only changes in the solution made during the experiment. Of course, the levels in the supply bottles were brought up to the original level by the addition of distilled water each day before the jars were flushed.

Addition of nitrogen. If nitrogen is added in large amounts at the beginning of the experiment, the seedlings will be killed by doses that may prove optimum if added over a longer period of time. To start with, the levels of nitrogen in p.p.m. were 0, 25, 50, 75, 125, 150, 200, 300, 400, and 500. These levels were built up gradually over a period of time extending from February 4 to March 2. For each application enough stock solution of NH_4NO_3 was added to the solution in the supply bottles to increase the concentration by 25 p.p.m. On some of the higher concentrations the additions were increased toward the end of the period. The application schedule is shown in table 2. The body of the table shows the number of applications required to build up the desired concentration for each of the levels of nitrogen. The levels

Table 2. Nitrogen Application Schedule—Sand Culture Experiment, February to April, 1939

Application date	Total nitrogen supplied in p.p.m.									
	0	25	50	75	125	150	200	300	400	500
February 4	......	25	25	25	25	25	25	25	25	25
February 8	......	......	25	25	25	25	25	25	25	25
February 11	......	......	......	25	25	25	25	25	25	25
February 15	......	......	......	......	25	25	25	25	25	25
February 18	......	......	......	......	25	50	50	50	50	50
February 21	......	......	......	......	......	......	50	50	50	50
February 23	......	......	......	......	......	......	......	100	100	100
February 27	......	......	......	......	......	......	......	....	100	100
March 2	......	......	......	......	......	......	......	....	....	100

of nitrogen will be expressed as total amount supplied and not as concentrations of that element, because the constant absorption by the seedlings continually reduces the concentration of nitrogen in the culture solution.

Some difficulty arose when the solution had to be replaced on March 27. A sample of the solution taken out of each of the bottles was analyzed for nitrogen and it was assumed that the concentration of the solution held by the sand would be the same. This may not have been accurate but it seemed to be the only thing to do under the circumstances. From these figures it was possible to determine the amount of nitrogen taken by the seedlings from the solution previous to March 27. An attempt was then made to add enough nitrogen to the fresh solution to bring each nitrogen level up to the original concentration. As a result of this procedure the total amount of nitrogen supplied to each of the 10 levels of nitrogen became: 0, 48, 95, 145, 230, 275, 336, 537, 702, and 855. These figures include the amount added with the new solution plus the amount the seedlings extracted from the original solution; hence the irregularity of the figures.

Solution pH value. It has been found by many workers that coniferous seedlings grow best in a soil with a *p*H value of 5.0 to 6.0. Studying the effect of the *p*H on the absorption of nitrogen, Davidson and Shive *(9)* found that NO_3 was absorbed best at *p*H 4 and ammonium best at *p*H 6. Mitchell *(18)* made several *p*H determinations on the solutions he used for his sand culture experiments and found that the *p*H values fell within this range and changed very little with the various levels of nitrogen. Only one *p*H determination was made during this experiment. This was made using a sample of sand from the upper one and one-half inches in each jar. The results of these tests are as follows:

P.P.M. of nitrogen supplied	pH	P.P.M. of nitrogen supplied	pH
0	5.5	275	5.6
48	5.5	336	5.5
95	5.6	537	5.6
145	5.7	702	5.7
230	5.7	855	5.8

This determination was made four weeks after the experiment was started. Although these values fall within the optimum range for jack pine and vary but little, *p*H tests should have been made frequently throughout the course of the study, for the contact exchange of ions explained by Jenny and Overstreet *(14)* may have played a far more important role than indicated by one *p*H determination. It is well known (Meyer and Anderson, *17)* that as a result of aerobic respiration in the root cells there is an accumulation of carbon dioxide near the root surface. This in turn forms carbonic acid by reaction with the water in the solution. The ionization of this acid may produce hydrogen ions absorbed on the root surface. These ions are probably exchanged for certain other nutrient ions found in the culture solution. As a result of this exchange the *p*H value of the solution may be changed. It is for this reason that one should be very careful to have the solution well buffered or else evaluate the influence of *p*H.

Planting seeds and care of seedlings. Variation in the size of individual seedlings within a given treatment has presented serious difficulties for many workers. Addoms *(2)* selected her stock by size and found that this was not satisfactory. Mitchell *(18)* weighed the seeds and calculated a correction factor to be applied to seedlings grown from different size seed. By the use of this factor he was able to convert all the seedling weights to those of seedlings from a given seed size since there is a direct relationship between the size of the seed and the size of the seedling.

In this experiment, jack pine seed obtained from the Lake States Forest Experiment Station was screened to remove the small seeds and run through a blower to remove the light ones. The seeds were then weighed individually on an analytical balance and divided into 1.0 mg. weight classes. It was found that most of the seeds fell in the 2.5-3.5 and 3.5-4.5 classes. The first class will be referred to as number 3 seeds and the second as number 4 seeds. These seeds were then planted (January 9, 1939) in germinating flats filled with washed sand. During the period of germination they were watered with distilled water as needed. After the seedlings had lost their seedcoats, 25 were transplanted to each jar of the sand culture setup. This work was done February 4, after the jars had been flushed with the nutrient solution. In order to avoid the necessity for making corrections for seed size, seedlings from number 3 seeds were planted in all the levels of nitrogen in one replication and seedlings from number 4 in the other. In this manner, seed size was thrown in with replication effect and, as will be seen later, there was no significant difference in the seedlings which were grown from different size seed.

During the course of the experiment very few seedlings died and those that did were replaced by other seedlings which were marked and discarded in the final analysis. This was done to keep the same number of seedlings drawing from each jar.

Weather records. The thermostats in the greenhouse were set to go on whenever the temperature went below 70° F. On bright sunny days the temperature went up to 90° or 95° but unfortunately no temperature records were taken. However, records were taken in experiments to be discussed later. The relative humidity obtained from hygrograph records is expressed in day and night weekly averages in table 3. The evaporation rate was calculated by the use of Livingston atmometers. Two sets of one black and one white bulb each were used, but owing to the great variation between the two sets, the results were not averaged but

Table 3. Relative Humidity and Atmometer Records, Forestry Greenhouse, 1939

Date	Relative humidity		Evaporation in c.c. for atmometers					
	Weekly averages		Set number 1			Set number 2		
	Day	Night	Black	White	Difference	Black	White	Difference
February 6-12	38	37	366	356	10	405	382	23
February 13-19	45	43	317	294	23	430	385	45
February 20-26	43	44	390	362	28	456	385	71
February 27-March 5	53	52	326	290	36	378	333	45
March 6-12	50	55	333	309	24	392	333	59
March 13-19	44	46	366	338	28	430	365	65
March 20-26	44	63	354	304	50	392	331	61
March 27-April 2	40	62	328	288	40	386	305	81
April 3-9	44	54	311	275	36	354	305	49
April 10-16	46	59	296	247	49	323	272	51
April 17-23	52	66	239	209	30	303	226	77
April 24-30	40	56	427	370	57	503	418	85
Total	......	......	4,053	3,642	411	4,752	4,040	712

Total black	8,805 c.c.
Total white	7,682 c.c.
Difference	1,123 c.c.

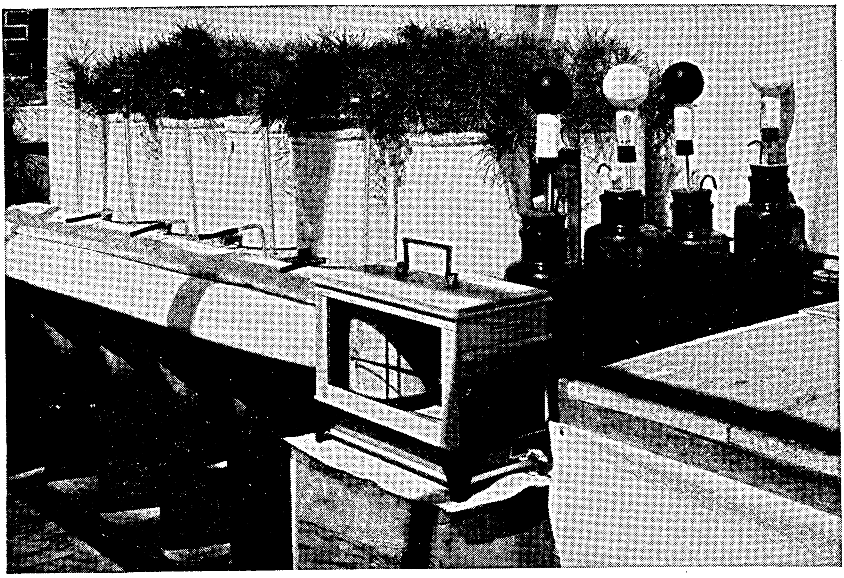


FIG. 2. Sand culture apparatus

are presented individually in table 3. The evaporation from set one, which consisted of the two atmometers centrally located (see figure 2), was consistently lower than that of set two, which included the two atmometers shown farthest apart. This difference may have been due to difference in circulation of air, reflection of light, or shadows. In comparing the atmometer records of this experiment with experiments to follow, the totals for the two black and the two white bulbs will be used.

Harvesting seedlings. At the end of the experiment the contents of each jar were washed out onto a screen by the use of a small stream of water. After the roots were thoroughly washed to remove all the sand, the seedlings were separated carefully to avoid root breakage. Ten seedlings for analysis were then selected randomly from each jar. Each seedling was cut in two at the root collar and the following measurements were obtained: stem height, root length, number of side shoots on the stem, and oven dry weight of stem and roots (dried at 100° C.). From these dry weights the root/shoot ratio was computed. These measurements were taken individually for each of the 200 seedlings used for the analysis. The dried samples were then sent to the Division of Agricultural Biochemistry for a nitrogen analysis. The nitrogen determined by the Kjeldahl method was expressed in percentage of dry weight.

RESULTS AND CONCLUSIONS

Effect of nitrogen supply on seedling growth. The dry weights of 83-day-old jack pine seedlings varied with the amount of nitrogen supplied. In figure 3 the jars containing the seedlings are

FIG. 3. Jars arranged according to nitrogen supplied

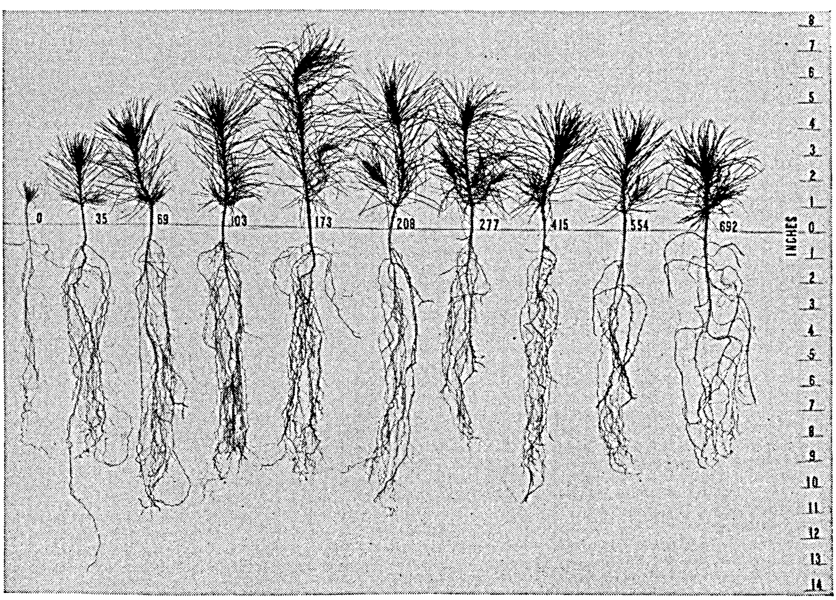


FIG. 4. Seedlings arranged according to nitrogen supplied

Code numbers on the pots and seedlings refer to the following concentrations of nitrogen in p.p.m.: 0=0; 35=48; 69=95; 103=145; 173=230; 208=275; 277=336; 415=537; 554=702; 692=855 shown arranged in order of the amount of nitrogen supplied. The numbers on the jars and seedlings are in code but the nitrogen concentrations in p.p.m. are given under figure 4. After the seedlings were washed from the jars, a representative seedling from each level of nitrogen was selected and arranged in order for the picture shown in figure 4. It will be readily apparent that the largest seedlings were supplied with about 230 p.p.m. of nitrogen. The averages for all the growth data taken are presented in table 4. Each average is based on 20 seedlings (10 from each replication). The nitrogen percentage, which will be discussed later, is also given. An analysis of variance was run on each set of measurements: the "F" values for nitrogen treatments and for replication (blocks) along with the standard deviation are given at the base of the table. The "F" value for treatment was highly significant in every case except two, that of the number of side shoots and weight of roots.

The average seedling weight (total weight as given in figure 6) was greatest at a nitrogen concentration of approximately 200 p.p.m. There was a rapid increase in weight from the control receiving no nitrogen up to the 230 p.p.m., and from this point on the curve dropped and leveled off. The same trend is also shown for stem weight (figure 6), and stem height (figure 5) which closely correspond to each other. These trends are in agreement with those of Mitchell *(18, 20)* except that he found that the optimum for white and Scotch pine was 300 p.p.m.

The weight of the roots (figure 6) increased very rapidly up to 100 p.p.m. Above this point the increase was very slight up to 200 p.p.m., beyond which there was a decrease and final leveling off of the curve. The fact that the roots had very nearly reached their largest weight at 100 p.p.m. indicates that the nitrogen requirement of the root is less than that of the stem. This may be important in nursery work because a well-developed root system is important in forest planting. It will be seen in figure 5 that the length of the roots bears out the same conclusion regarding the optimum for root growth. In the control plot there was not enough nitrogen for much growth, and the roots that did develop were very spindling, even though they were almost as long as some in the higher concentrations. When they received a small amount of nitrogen which was still below the optimum, they grew much longer than seedlings which were receiving the optimum supply or above.

The root/shoot ratio based upon weight shows a rapid decrease from 0 p.p.m. to 145 p.p.m. (figure 7). From that point on

Table 4. Summary of Treatment Means† and Analysis of Variance—Sand Culture Experiment, February to April, 1939

Nitrogen treatment	Stem height	Root length	Side shoots	Mean weight Total	Mean weight Stem	Mean weight Root	Root/shoot ratio	Nitrogen percentage Stems	Nitrogen percentage Roots
	Inches	Inches	Number	Mg.	Mg.	Mg.		Per cent	Per cent
0	1.04	12.48	0.0	54.93	25.45	29.48	1.168	1.02	0.86
48	2.00	14.92	1.7	388.62	203.30	185.32	0.914	1.82	1.20
95	3.64	12.40	1.9	674.54	424.39	250.14	0.590	2.02	1.43
145	4.54	12.00	2.4	836.89	571.88	265.01	0.466	2.06	1.46
230	4.82	11.58	2.2	909.28	639.10	270.18	0.426	2.74	2.10
275	3.78	12.06	1.5	750.47	511.72	238.74	0.464	3.20	2.30
336	3.94	12.02	1.8	782.98	540.82	242.16	0.452	3.32	2.62
537	3.54	10.98	2.0	742.18	522.08	220.10	0.421	3.36	3.26
702	3.32	11.36	1.9	742.98	531.68	211.30	0.403	3.77	3.44
855	2.98	11.80	1.6	752.58	509.80	242.78	0.477	4.19	3.50
"F" value treatment	37.09**	5.08*	3.65*	45.29**	61.86**	9.33**	29.81**	178.65**	6.24**
"F" value blocks	3.59	1.50	3.27	-	3.87	4.24	8.04*	32.74**	1.01
S.D.	0.26	0.67	0.21	168.53	33.90	32.44	0.066	0.11	0.55

† The means are based on 20 seedlings each.
* Significant (analysis of variance).
** Highly significant (analysis of variance).

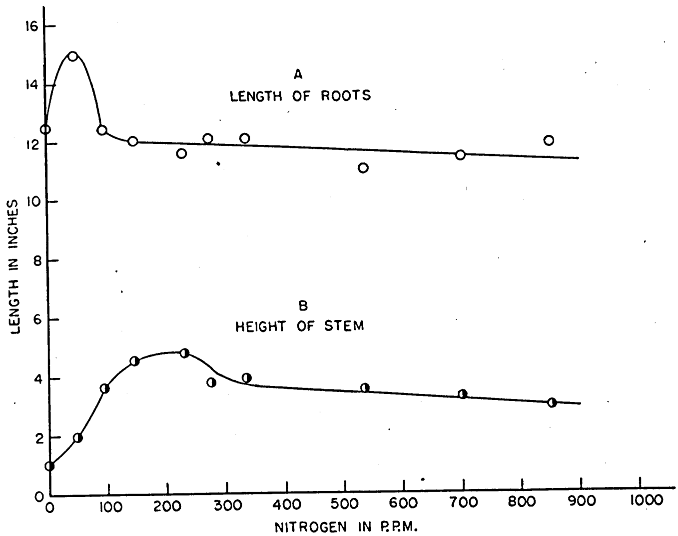


FIG. 5. Root length and stem height for jack pine seedlings grown in sand culture with varied nitrogen supply

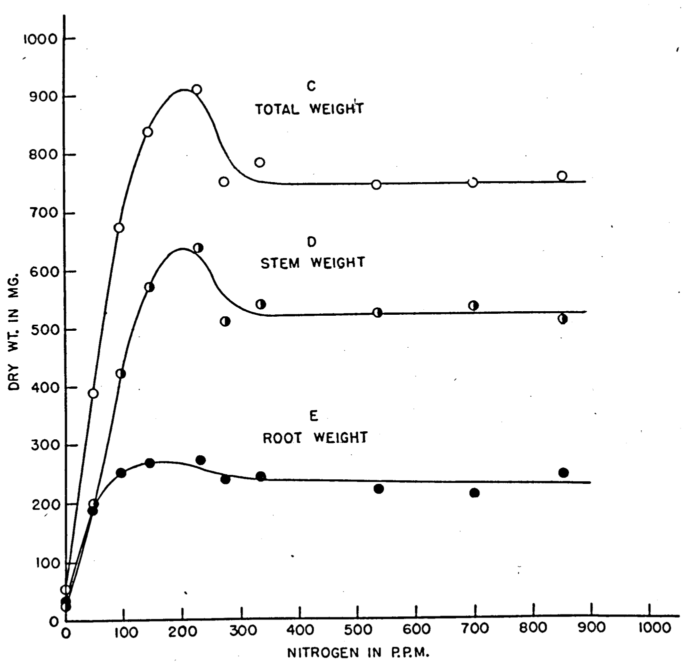


FIG. 6. Total weight, stem weight, and root weight for jack pine seedlings grown in sand culture with varied nitrogen supply

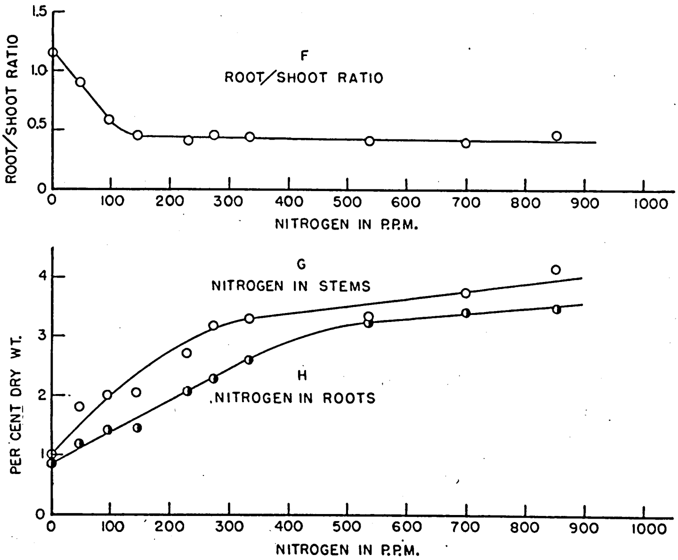


FIG. 7. Root/shoot ratio, per cent of nitrogen in the stems, and per cent of nitrogen in the roots for jack pine seedlings grown in sand culture with varied nitrogen supply

there was no change in the root/shoot ratio. This was confirmed by a correlation coefficient of 0.119 found for the straight portion of the curve. This would indicate that as soon as the roots have received enough nitrogen for optimum growth there is little or no change with increase in available nitrogen. This decrease in root/shoot ratio with increase in nitrogen can be explained in the following manner (Meyer and Anderson, *17*). If the nitrogen concentration in the substratum in which the plants are growing is low, most of the nitrogen is utilized in the synthesis of amino acids in the roots, the carbohydrates used for this synthesis being translocated downward from the leaves. Since the roots are deficient in growth substance most of the amino acids are used in the synthesis of protoplasmic proteins during the growth of the roots.

As a result only a comparatively small portion of the nitrogenous compounds escape utilization in the roots and is translocated to the stem. The stems are therefore deficient in proteins and hence the high root/shoot ratio. On the other hand, when the supply of nitrogen is large, a sufficient quantity will be translocated to the stems and the root/shoot ratio will decrease. If, however, conditions are such that the supply of carbohydrates is low, another serious maladjustment will arise. In this case the limited quantity of carbohydrates will be used up in the stem since there is plenty of nitrogen, and little carbohydrate will be left over for the roots. This results in a still further decrease in the root/shoot ratio and points out the need for well-balanced growing conditions.

The number of side shoots on the stem was another measurement of growth taken on the seedlings. Seedlings receiving no nitrogen developed no side shoots. The "F" values (table 4) are based upon all the levels of nitrogen other than this control treatment. The significant "F" value for treatment would indicate that there is probably a relationship between the number of side shoots and the nitrogen supply, the seedlings receiving the optimum supply having the greatest number of shoots.

Effect of nitrogen supply on nitrogen content of seedlings. With the increase in the nitrogen supply there is an increase in the nitrogen content of the seedlings expressed in percentage of dry weight. The results of this experiment show the relationship to be curvilinear (see table 4 and graphs G and H, figure 7). If we consider only the portion of the curve from 0 to 300 p.p.m., which would be sufficient for all practical purposes, a straight line could probably be fitted to the data without much error. The nitrogen percentage of both the roots and shoots follows relatively the same trend, that of the roots being somewhat lower. This relationship suggests a method of determining the available nitrogen in the soil. Since at the optimum supply of nitrogen (200 p.p.m.) the seedlings contained 2.75 per cent of that element, it seems that a seedling with nitrogen content of 2.75 per cent should have grown in the most favorable nitrogen supply. This would be true only when the seedling had received a sufficient supply of all other nutrients, for, in many cases, if some other nutrient is insufficient, this relation of internal and external nitrogen is changed. For example, Beckenboch *(4)* finds an inverse relationship of internal and external nitrogen when potassium is lacking in the nutrient solution. It would be necessary to grow seedlings in the soil to be tested after a sufficient quantity of all other elements with the exception of nitrogen had been added. An analysis of these seedlings and interpretation of graph G in figure 7 would tell one how far the supply of nitrogen in the soil was below the optimum. The curve for nitrogen percentage in the stems agrees quite closely to that presented by Mitchell *(20)*, indicating that a seedling, whether jack pine or white pine, growing in a given concentration of nitrogen will have a corresponding nitrogen percentage. For example, white pine seedlings grow best in 300 p.p.m. of nitrogen and have 3.26 per cent nitrogen. Jack pine seedlings, although they do not grow best at 300 p.p.m., have a nitrogen percentage of about 3.25 per cent (graph G, figure 7, page 17).

It will be seen in table 4 that the "F" value for blocks in the case of nitrogen percentage in the stems is highly significant. The nitrogen percentage in all treatments for the block in which seedlings originated from number 4 seeds was consistently lower than that for number 3 seeds. Since block effect cannot be separated from seed size no conclusions can be drawn. In the case of nitrogen percentage in the roots the "F" value for blocks was not significant.

There is one other point that should be mentioned in connection with the internal and external nitrogen relationship. If nitrogen is held constant and seedlings are stunted in growth by lack of light or other causes, the percentage of nitrogen in those seedlings will be increased over seedlings not so stunted (see Mitchell, *18*). For this reason, in certain cases it may be more advisable to use total nitrogen expressed on a weight basis. In so doing some of this difficulty could be avoided. However, in this experiment nitrogen percentage worked very well.

Effect of nitrogen supply on seedling color. The leaves of the seedlings receiving no nitrogen were a yellow-green color. As the supply of nitrogen was increased, the color became a deeper green, almost a blue-green at the upper part of the range. There was one exception to this trend; in the higher nitrogen concentrations a few of the seedlings turned yellow toward the end of the experiment. As for example, in jars receiving 855 p.p.m. all the leaves of two or three seedlings in each jar turned yellow. All the rest of the seedlings had a deep green color. The only explanation for this situation seems to be that the chlorophyll is in some manner destroyed as a result of the high nitrogen concentration. This same condition was reported by Chapman *(6)* in 1933.

DISCUSSION

In this experiment the growth and nitrogen content of the seedlings have been correlated with total nitrogen supplied and not with the nitrogen concentration of the culture solution. When the solution was changed on the 52nd day of the experiment because of the growth of algae in the supply bottles, a nitrogen analysis was made on a sample from each nitrogen level. It was found that the higher levels had been depleted more rapidly than the lower levels of nitrogen. For example, the solution in the jars receiving 500 p.p.m. during the first month of the experiment had been depleted down to 225 p.p.m., while the solution containing originally 75 p.p.m. had been depleted to about 6 p.p.m. At the end of the experiment another analysis of the solutions was made and about the same rates of depletion occurred. It is therefore apparent in a sand culture experiment of this kind that the seedlings grow in the original solution for but a short time at the beginning of the experiment. In this case, however, the nitrogen levels were built up gradually during the first part of the experiment so not even this condition was true. It is for this reason that the nitrogen treatment is expressed in total supplied.

An attempt was made to account for the nitrogen taken from the culture solution by making a nitrogen analysis of the sand in the jars and converting this in terms of p.p.m. in the solution held by the sand. These figures were averaged with the culture solutions remaining in the supply bottles and the results in turn compared with the total amount of nitrogen supplied. The samples of sand had to be taken from the pots before the seedlings were washed out and because of the danger of breaking the roots it was impossible to obtain a representative sample. This difficulty, along with other things that could not be accounted for, as for example the growth of bacteria and algae on the surface of the sand, made it impossible to trace the depletion of nitrogen accurately. Evidence, however, indicates that the sand holds considerably more nitrogen in relation to the amount of solution it holds than does the solution in the supply bottles. This means that sand acts more than just as a means of holding plants and until a satisfactory method of tracing the depletion of nitrogen is found, about the only thing that can be done is to correlate growth with total supplied.

Even with these disadvantages of the sand culture technique used in this experiment it is still a very good method of testing the nutritional needs of tree seedlings. The first and most important point in favor of the use of this system is that the seedlings do grow very well. The second thing in its favor is that the roots grow in a more natural environment than those growing in a solution culture. One other important consideration is the problem of aeration. In this type of experiment the problem is automatically solved, but in solution cultures it often presents serious difficulties. Although the design used in this experiment has certain disadvantages, they are considerably outweighed by its advantages.

A Pot Culture Test of Cloquet Nursery Soil

The first step in the successful application of a nitrogen fertilizer in the nursery is the determination of the nitrogen deficiency of the soil in question. There are many methods available but the pot culture method was chosen in this case. The experimental work for this study was carried out during the summer of 1939 at the Cloquet Forest Experiment Station, Cloquet, Minnesota. This investigation seemed to fall in a logical sequence with the one reported in the first part of this bulletin, for after the fundamental relationships of nitrogen supply and growth are understood for a given species, the next thing is the practical application of that knowledge to nursery practice. In this application the first step is the determination of the amount of available nitrogen in the soil, and after that one can turn to methods of supplying the amount of nitrogen needed.

EXPERIMENTAL METHOD

Pot culture apparatus and soil used. The apparatus used in this experiment was exactly the same as that used for the sand culture study. Since the experiment was carried on out-of-doors it was necessary to use a different sort of box to hold the jars. Figure 8 gives a general view of the arrangement. The sides of

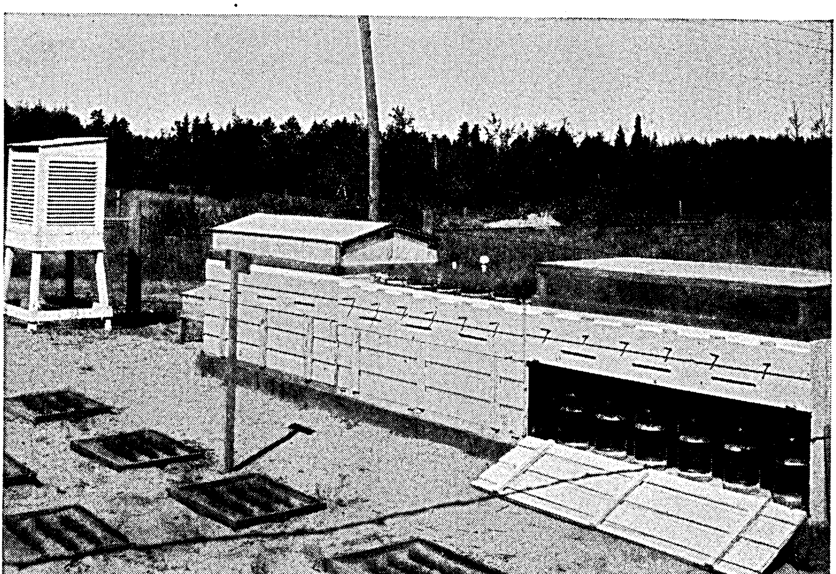


FIG. 8. Pot culture apparatus

the boxes were built up to the top edge of the jars so that the jars could be packed in moist sphagnum moss to prevent their surfaces from being heated by the sun. The supply bottles were tightly enclosed in the lower part of the box. One door was removed when the picture was taken. Because of the danger of damage by birds and rodents it was necessary to place screens over the jars. At the right of the picture one of the screens is shown in place. The occurrence of rain offered another problem. This was solved by the use of cello-glass covers which were placed on the boxes at night and during the day whenever it rained. One of those covers is seen to the left of the picture.

Instead of putting washed sand in the jars as was done in the sand culture experiment, nursery soil was used (Omega loamy fine sand). Samples of nursery soil taken at random over the nursery were thoroughly mixed and screened. After a layer of coarse gravel was placed in the bottom of each of the jars to facilitate drainage, they were filled with the prepared soil.

Culture solution. As was stated in the first part of this bulletin, in order to determine the available nitrogen in a soil using this technique, it is necessary to supply optimum concentrations of all other nutrients except nitrogen. This presented a difficult problem because nothing was known as to the supply of those nutrients in the nursery soil. In order to avoid the use of too strong a concentration of the standard solution, it was decided to use a solution of one half that used for the sand culture experiment. This was purely a chance selection and only by further experimentation would one be able to determine the exact strength that should be used for such a purpose. The concentration of the standard solution used is given in table 5.

Addition of nitrogen. According to Mitchell *(18)* the available nitrogen in a soil can be determined by only one treatment, the control receiving no nitrogen. From a nitrogen analysis of the seedlings grown with only soil nitrogen, but supplied with all other nutrients, the available soil nitrogen in terms of p.p.m. can

Table 5. Standard Culture Solution—Pot Culture Test of Cloquet Nursery Soil, Summer of 1939

Source	P.p.m. of source	Mg. for 3½ L.	Concentration of nutrient element, p.p.m. P	K	Ca	S	Fe	Mg
KH_2PO_4	278.0	973.0	63.4	79.8	....	....	.....	....
$MgSO_4.7H_2O$	445.0	1,557.5	....	....	....	57.9	.....	43.9
$CaCl_2.2H_2O$	333.8	1,168.1	....	....	91.0	....	.....	....
Iron citrate	5.0	17.5	....	....	....	....	0.8	....

be interpreted from a graph similar to graph G in figure 7. Possibly soil nitrogen does not behave the same as nitrogen supplied in the form of NH_4NO_3. If this is the case there may be some error involved in this method. In order to obtain evidence for or against this idea, five levels of nitrogen were used (10, 50, 100, 150, and 200 p.p.m.) in a randomized block experiment with two replications. The highest concentration was set at 200 p.p.m. because that amount gave optimum growth in the sand culture experiment. If more were added an overdose of nitrogen would result. A pot receiving no nitrogen should have been included in order to obtain an accurate check on natural soil nitrogen present.

After 3½ liters of standard culture solution was placed in each supply bottle, the total amounts of nitrogen for each level were added to the appropriate bottles. The jars were then flushed, allowed to drain for one day, and marks placed at the upper limit of the solution in the supply bottles. In order to dilute the solution to allow the seedlings a better chance to become accustomed gradually to the nitrogen present, one liter of distilled water was placed in each supply bottle. After the jars were flushed again, the seedlings were transplanted into them. It took about four weeks for the evaporation from the surface of the soil and the transpiration from the seedlings to reduce the solution to its original volume. In this manner the concentration of the solution was gradually increased. This may not have been true, however, for because of the chemical nature of soil the retention of nitrogen may have been different than one would expect. Conrad *(7)* studied the retention of various forms of nitrogen by soils known to be deficient in that element and found that nitrate ions were not retained by the soil but ammonium ions were.

It was soon discovered that the water-holding capacity of the nursery soil was much greater than that of the washed sand used previously. Instead of flushing the jars once a day, once a week was sufficient. Although the soil retained sufficient moisture to last a week, the amount of aeration was not so great as that in the sand culture experiment.

Planting seeds and care of seedlings. Seeds in the 3 mg. class were used for all the jars. The germination of the seed and care of the seedlings were exactly the same as that for the sand culture experiment. On June 15, 30 seedlings were transplanted in each of the jars and on September 8 they were harvested in the same manner as described in part one of this bulletin. This gave the seedlings 84 days to grow.

Weather records. The weekly averages of the maximum and minimum temperature and of day and night relative humidity are given in table 6. The evaporation was again measured by the use of Livingston atmometers. Two sets of one black and one white bulb each were used. The results are also shown in table 6.

RESULTS AND CONCLUSIONS

Seedling mortality was found to be quite closely related to the amount of nitrogen supplied, the greatest mortality occurring at the highest concentration of nitrogen (see table 7). From the seedlings that survived, a random sample of 10 was taken from each jar for analysis. Before the seedlings were washed out of the soil, the jars were arranged in order of the p.p.m. of nitrogen received (see figure 9).

A summary of the means for each of the growth measurements and for the percentage of nitrogen in the stems and roots is given in table 8. An analysis of variance was run on each set of the original measurements. The "F" values and standard deviation are given at the base of the table. Of the growth measurements only the "F" value for weight of stems and root/shoot ratio reached the 5 per cent level of significance. A graph of each of these measurements is shown in figure 10. It is readily apparent (graph D) that the seedlings receiving 100 p.p.m. of nitrogen attained the greatest weight, but since the odds are only slightly better than 20 to 1 that the treatment differences are real

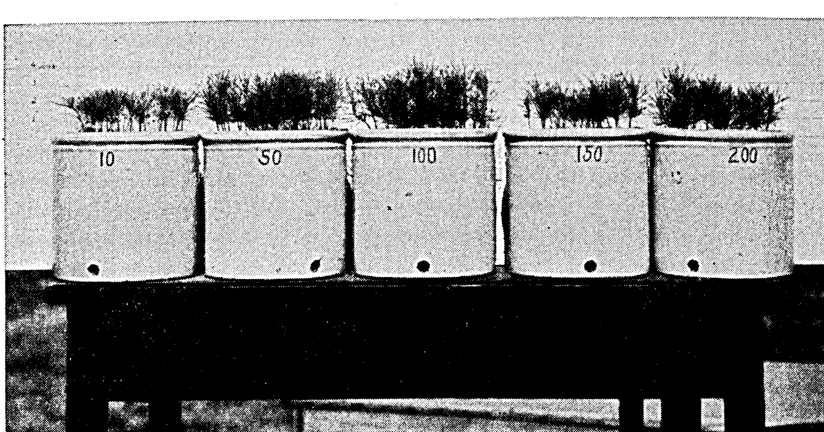


FIG. 9. Pot culture test of Cloquet nursery soil
Jars arranged in order of nitrogen supplied in p.p.m.

Table 6. Temperature, Relative Humidity, and Atmometer Records, Cloquet Forest Experiment Station, 1939

Date	Temperature, weekly averages		Relative humidity, weekly averages		Evaporation in c.c. for atmometers					
					Set number 1			Set number 2		
	Maximum	Minimum	Day	Night	Black	White	Difference	Black	White	Difference
June 20-26	74	51	......	......	260	186	74	266	179	87
June 27-July 3	78	55	73	81	325	218	107	322	227	95
July 4-10	87	57	57	81	423	309	114	429	317	112
July 11-17	80	50	50	75	353	270	83	358	268	90
July 18-24	77	53	57	79	336	248	88	343	253	90
July 25-31	87	52	49	78	416	315	101	409	305	104
August 1-7	83	52	53	80	364	296	68	371	292	79
August 8-14	74	52	66	84	234	166	68	223	162	61
August 15-21	81	55	62	85	282	203	79	284	204	80
August 22-28	75	48	59	80	223	154	69	223	145	78
August 29-September 4	78	57	64	80	196	139	57	201	136	65
September 5-11	66	45	68	80	143	101	42	151	87	64
Totals	......	......	......	......	3,555	2,605	950	3,580	2,575	1,005

Total black	7,135 c.c.
Total white	5,180 c.c.
Difference	1,955 c.c.

Table 7. Mortality in Pot Culture Test of Nursery Soil—Summer, 1939

Nitrogen in p.p.m.	Total mortality through season	
	Block 1	Block 2
10	3	2
50	4	2
100	2	5
150	4	7
200	9	11

differences, the evidence seemingly apparent in graph C cannot be relied upon too strongly. In the sand culture experiment it was found that the jack pine seedlings attained the greatest weight when supplied with approximately 200 p.p.m. of nitrogen. Since the seedlings in this nursery soil pot culture grew best when they received 100 p.p.m. it would mean that the soil supplied the equivalent of 100 p.p.m. If this is true we are dealing only with that portion of the sand culture curve (graph D, figure 6) between 100 p.p.m. and 300 p.p.m. If we compare the graph for root length, stem height, root weight, and root/shoot ratio with the 100-300 p.p.m. range of the appropriate sand culture curve, a favorable resemblance will be seen. For example, the rapid change in the trend of root/shoot ratio (figure 7) occurs around 100 p.p.m. and from that point on there is very little change. The curve for root/shoot ratio for the seedlings growing in the nursery soil fell along the latter part of this curve and therefore was almost a straight line.

There proved to be in this pot culture test a very highly significant difference between the nitrogen supplied and the percentage of nitrogen in the seedlings. The percentage of nitrogen in the stems and roots was very nearly the same in each of the five levels of nitrogen (see table 8). Because of this similarity, the percentages in the roots and stems were averaged. Those averages are shown in graph G, figure 10. At the optimum nitrogen concentration of 100 p.p.m. the seedlings contained 2.17 per cent nitrogen. Now if we should determine from the nitrogen percentage graph in the sand culture experiment the p.p.m. of nitrogen corresponding to 2.17 per cent, we would obtain the total amount of nitrogen supplied (soil plus solution in pot culture). Since the nitrogen percentage graph for the pot culture was based on averages of roots and stems, the sand culture data were converted to the same basis. The relation of nitrogen supply to nitrogen content of the whole seedling is shown in figure 11. Mitchell *(18)* found that such a graph could be used for prediction

Table 8. Summary of Treatment Means† and Analysis of Variance—Pot Culture Test of Nursery Soil, Summer, 1939

Nitrogen treatment	Stem height	Root length	Side shoots	Mean weight: Total	Mean weight: Stem	Mean weight: Root	Root/shoot ratio	Nitrogen percentage: Stems	Nitrogen percentage: Roots
	Inches	Inches	Number	Mg.	Mg.	Mg.		Per cent	Per cent
10	1.98	9.47	1.00	159.00	98.80	60.20	0.608	1.22	1.28
50	2.50	10.20	2.20	246.42	166.43	79.99	0.478	1.80	1.84
100	2.60	8.85	1.80	254.54	185.66	68.90	0.372	2.21	2.13
150	2.22	8.31	1.55	196.19	143.48	52.68	0.353	2.22	2.30
200	2.14	8.72	1.65	221.94	161.00	60.98	0.376	2.42	2.36
"F" value treatment	2.46	1.38	6.58*	3.52	8.49*	1.07	8.48*	22.52**	34.95**
"F" value blocks	......	......	......	......	......	......	......	......	......
S.D.	0.23	0.89	0.24	29.42	15.95	14.15	0.052	0.14	0.11

† The means are based on 20 seedlings each.
* Significant (analysis of variance).
** Highly significant (analysis of variance).

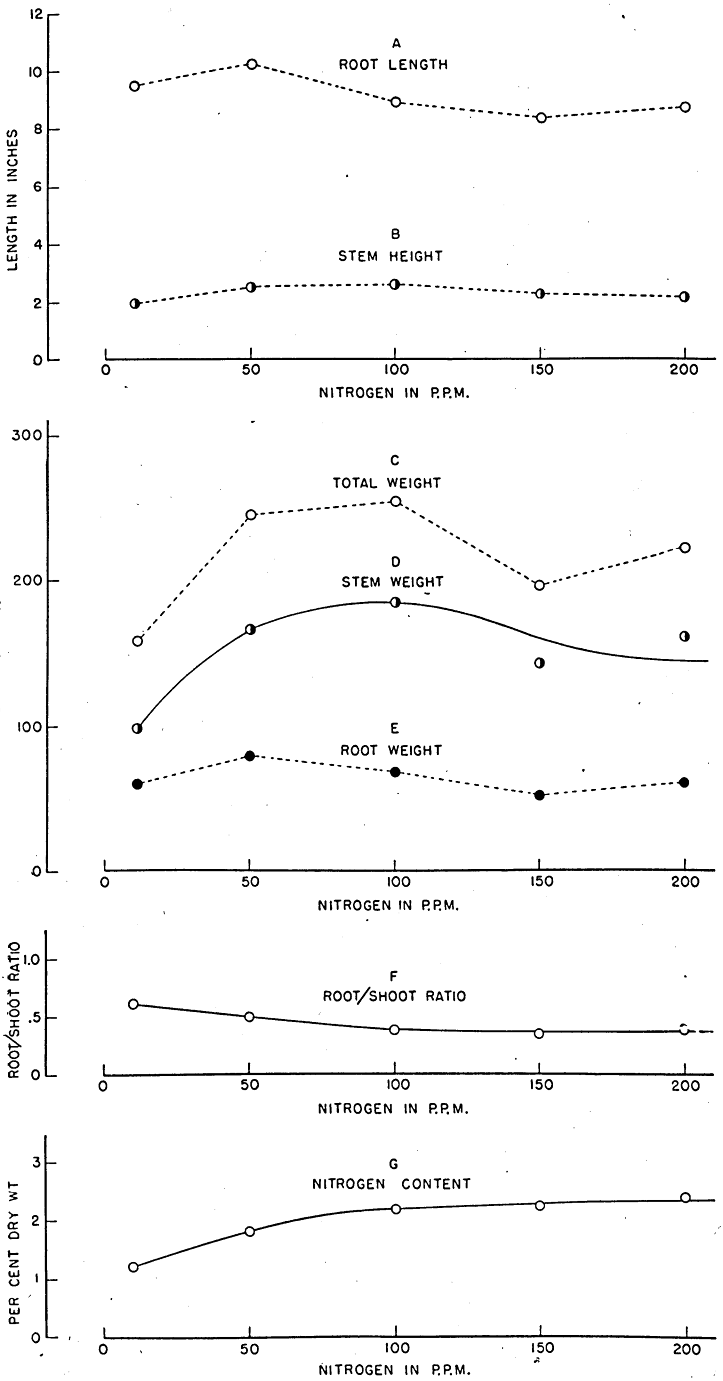


FIG. 10. Root length, stem height, total weight, stem weight, root weight, root/shoot ratio, and per cent of nitrogen in both roots and stems, for seedlings grown in Cloquet nursery soil with varied nitrogen supply

Break in each base line represents amount of nitrogen supplied by the soil.

of either variable with equal accuracy. There is no need of extending this graph beyond 300 p.p.m. because that nitrogen supply is well over the optimum and this would not be encountered in natural soils. For the range used, a straight line fits reasonably well. By interpolation on the graph or using the formula it will be seen that 2.17 per cent corresponds to 189 p.p.m. Since 100 p.p.m. had been supplied, that would leave 89 p.p.m. supplied by the soil. This agrees reasonably closely with the results using the weight of the seedlings. This would mean that there is roughly 40 to 50 per cent as much nitrogen in the soil as there should be. This method is open to criticism since it assumes that the soil not only retains nitrogen in the same manner as does washed sand but that the natural nitrogen held by the soil is absorbed at the same rate as is the nitrogen supplied in the form of NH_4NO_3. This criticism is probably correct and realizing it Mitchell *(18)* says the only reliable figure is the relative comparison between the nitrogen in a soil and the sand culture optimum. These proportions prove to be the same even though the rates of increase in nitrogen percentage may be different in sand and in soil. This is true with increase in available nitrogen, however, only when both relationships are linear. This relationship for the pot culture (graph G, figure 10) can hardly be considered linear; thus the method is not accurate but a reasonable approximation.

The data indicate that there is a relationship between the amount of nitrogen supplied to a soil and the efficiency of the seedlings in extracting the nitrogen already there. Table 9 brings out this point. In columns one and two are given the nitrogen supplied and corresponding nitrogen percentages based on dry weight for the pot culture experiment. In column three is given the p.p.m. interpreted from figure 11 (sand culture curve) for each of the nitrogen percentages in column two. These figures should then be equivalent to the total amount of nitrogen available in both the soil and solution. Now if the amount of nitrogen supplied by the solution is subtracted from these figures, the amount supplied by the soil should be obtained.

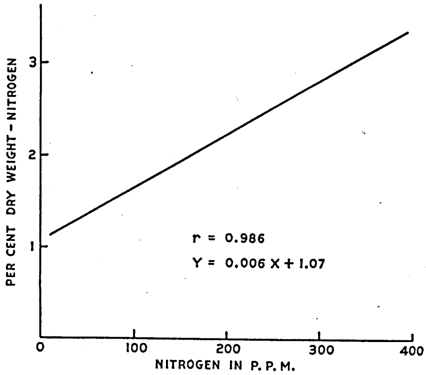


FIG. 11. Relation of nitrogen supplied to nitrogen content

Data based on sand culture experiment

Table 9. Relation of Nitrogen Supplied to the Use of Nitrogen Already in the Soil

Pot culture—nitrogen	Pot culture—nitrogen	Sand culture—nitrogen supplied, corresponding to each nitrogen per cent in pot culture	Nitrogen supplied by soil
p.p.m.	Per cent	p.p.m.	p.p.m.
10	1.25	31	21
50	1.82	129	79
100	2.17	189	89
150	2.26	205	55
200	2.39	227	27

These figures are given in column 4. The largest amount of soil nitrogen was available when the seedlings were supplied with just enough nitrogen to give optimum growth (100 p.p.m.). The amount of soil nitrogen available decreased on both sides of this point.

DISCUSSION

The seedlings attained a much larger size when grown in the sand culture than when grown in the pot culture using nursery soil. There were many differences in the environment of the seedlings grown in these two experiments that may help to account for these differences. First of all, as has already been mentioned, the nursery soil was difficult to use in the sand culture apparatus because of its fine texture. The seedling roots were probably not aerated properly. There was also considerable atmospheric difference during the two experiments. The relative humidity was considerably higher during the summer, when the pot culture test was made, than it was during the early spring, when the sand culture experiment was carried on (see tables 3 and 6). The atmometer records show considerable differences in the evaporation during the two experiments. The total evaporation was much less during the pot culture experiment but the difference between the black and white bulb was considerably greater (see tables 3 and 6). The sand culture seedlings received only about 57 per cent as much light as did the pot culture seedlings. This percentage is based upon the difference in the evaporation from the black and white bulbs, the black having the higher evaporation owing to absorption of heat from the sun's rays. This comparatively low light during the sand culture was probably somewhat compensated for by the higher air temperature in the greenhouse. In the greenhouse the temperature never went much below 68° F. but during the pot culture test the temperature dropped as low

Table 10. Nitrogen Analysis of Solution and Soil—Pot Culture Test of Nursery Soil, Summer, 1939

Nitrogen in culture solution		
At beginning of experiment	At end of experiment	Nitrogen in soil
p.p.m.	p.p.m.	Per cent
10	2	0.059
50	4	0.076
100	10	0.080
150	7	0.096
200	12	0.101
Untreated nursery soil	......	0.058

as 46° to 56° F. every night. This no doubt severely curtailed growth.

In this experiment an attempt was again made to determine the amount of nitrogen taken from the culture solution and the amount accumulated by the soil. Table 10 gives the results of these determinations. All of the solutions were fairly well depleted but the percentage of nitrogen in the soil was directly related to the amount supplied at the beginning of the experiment.

Nitrogen and Drouth Resistance

Before the results of the previously discussed experiments will have practical application, the effect of nitrogen on the drouth resistance of jack pine seedlings must be known. This was the purpose of the two greenhouse experiments reported below. The first, a small experiment with five levels of nitrogen, was designed to determine the effect of a wide range in nitrogen supply on the drouth resistance. The second had 10 levels of nitrogen all within a relatively narrow range. The object of this investigation was to study in detail the levels of nitrogen ranging from 50 to 500 p.p.m. A secondary objective for both of these studies was to gain additional evidence concerning the effect of nitrogen supply on the growth and the nitrogen content of jack pine seedlings. These two experiments will be referred to as drouth experiment numbers one and two.

DROUTH EXPERIMENT NUMBER ONE, SPRING, 1940

Experimental Method

Sand culture apparatus. The same sand culture apparatus was again used for this experiment. The sand used was from the same source as that used in the first sand culture experiment.

Culture solution. The concentration of the standard culture solution was the same as that for the first experiment given in table 1. After 3½ liters of this solution was placed in each of the supply bottles on January 28, 1940, the jars were flushed and allowed to drain 24 hours before the marks were placed at the upper level of the solution in the supply bottles. Then a liter of distilled water was added to each, making a total of 4½ liters in each system. In this way the solution was made more dilute for the young seedlings. The concentration was gradually increased by allowing the evaporation from the surface of the sand and the transpiration from the seedlings to reduce the solution to its original volume.

On March 16, the second application of culture solution was made. This application increased the concentration of the solution by one half its original strength. The application was made by the addition of a concentrated stock solution to each of the bottles.

Addition of nitrogen. The rate of depletion of nitrogen from the culture solution was determined both at the middle and end of the 1939 sand culture experiment reported earlier in this bulletin. By adding nitrogen in the experiment run in the spring of 1940 according to the rates of depletion of the 1939 experiment, the author attempted to hold the nitrogen concentration at the following levels: 0, 75, 150, 200, and 500 p.p.m. During the first 35 days of the study, these levels were built up following an application schedule similar to that in table 2. During this period some of the nitrogen was used so at the end of 35 days enough nitrogen had to be added to make up for this depletion. From that time on the nitrogen was added as it was depleted. These depletion rates, of course, were based on the 1939 experiment. At the end of the experiment the jars had received the following levels of total nitrogen supplied: 0, 170, 325, 340, and 905 p.p.m. This gave a wide range of nitrogen levels for a preliminary drouth resistance study.

Planting seeds and care of seedlings. The seeds were weighed and germinated in the same manner as they were in the first sand culture experiment. The experiment was designed in the form of a randomized block with five levels of nitrogen and two replications. On January 29, seedlings from 3 mg. seeds were transplanted in replication one and those from 4 mg. seeds in replication two. Because of good germinating condition it was possible to do this transplanting only 13 days after the seeds had been planted. At the end of the experiment the analysis of variance showed that in every case the difference between blocks was

Table 11. Atmometer Records, Forestry Greenhouse, 1940

Date	Evaporation in c.c. for atmometers					
	Set number 1			Set number 2		
	Black	White	Difference	Black	White	Difference
January 29	Atmometer bottles filled					
February 5	319	301	18	355	321	34
February 12	398	364	34	450	375	75
February 19	301	290	11	335	304	31
February 26	442	430	12	468	434	34
March 4	337	312	25	385	330	55
March 11	333	289	44	372	301	71
March 18	358	326	32	405	334	71
March 25	479	426	53	538	408	130
April 1	370	311	59	395	325	70
April 8	284	246	38	310	256	54
April 15	415	329	86	428	341	87
April 22	383	306	77	394	340	54
Total	4,419	3,930	489	4,835	4,069	766

Total black	9,254 c.c.
Total white	7,999 c.c.
Difference	1,255 c.c.

not significant, indicating that as far as this experiment was concerned there was no difference in seedlings from the two sizes of seed. Twenty-two seedlings were placed in each jar. There were no transplanting mortalities and no replacements were necessary throughout the course of the experiment.

Weather records. The only weather record taken during this experiment was the atmometer record given in table 11. If tables 3 and 11 are compared it will be noted that the 1939 experiment received very nearly the same amount of light as the 1940 experiment.

Results and Conclusions

Effect of nitrogen supply on drouth resistance. On the 80th day the jars were flushed for the last time. An average seedling was selected from each of the jars and photographed (figure 12). The jars were then allowed to stand in the greenhouse for three days, and on the morning of April 20 the glass tubes (connecting the jars and supply bottles) were removed. Stoppers were placed in the holes at the base of the jars and at 8:30 a.m. the jars were randomly arranged in the drouth machine. The drouth machine was similar to the one described by Shirley *(24)*. This machine consists of a rotating platform enclosed in a cylindrical chamber about 5 feet in diameter. The platform rotates about two revolutions per minute. The temperature, controlled by an automatic heating device, was set at 94° F. The humidity was controlled by pumping dry air from a cold room into the chamber. This pump

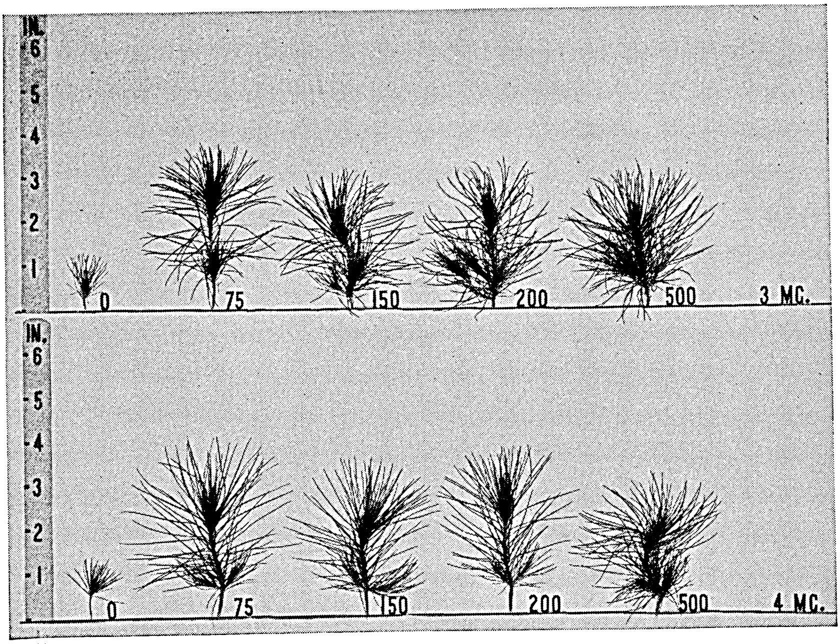


FIG. 12. Seedlings arranged according to nitrogen supplied

Code numbers at base of seedlings refer to the following concentrations of nitrogen in p.p.m.: 0=0; 75=170; 150=325; 200=340; 500=905

was controlled by a wet bulb thermoregulator. A relative humidity of 36 per cent was maintained by setting these two thermoregulators with the proper difference between them. The humidity fluctuated somewhat at first until the jars became fairly well dried out; then it ran quite constant. Four 200 watt mazda lights were on continuously in the machine.

Counts were made every two hours at first, and later every four hours day and night. The number of seedlings showing injury and number dead were recorded at each reading. The first injury noted was on the seedlings receiving no nitrogen. The tips of the needles started to turn brown. A seedling was considered dead when 75 per cent of the needles turned brown or became brittle; injury was recorded at the first sign of browning or wilting. In contrast to the needle browning of the seedlings receiving no nitrogen, the first sign of injury in the seedlings receiving nitrogen was the wilting of the tips of the seedlings.

The first three columns in table 12 give the results of the drouth test. Column one gives the average number of hours before injury was noted; column two, the average number of hours

Table 12. Summary of Treatment Means† and Analysis of Variance—Drouth Experiment Number One, February to April, 1940

Nitrogen treatment	Drouth resistance			Stem height	Mean weight			Root/shoot ratio	Nitrogen in stem
	Time to injury	Time to death	Time between injury and death		Total	Stem	Root		
	Hours	Hours	Hours	Inches	Mg.	Mg.	Mg.		Per cent
0	138	232	94	1.40	56.68	28.06	28.62	1.021	2.25
170	188	244	56	3.83	352.73	257.32	95.41	0.367	3.02
325	180	226	46	3.67	340.96	262.89	78.06	0.298	3.38
340	201	254	53	3.72	349.72	264.56	85.16	0.326	3.40
905	121	153	32	3.18	309.80	258.18	51.62	0.200	5.82
"F" treatments	16.20**	48.74**	9.25*	43.65**	32.51**	41.51**	7.75*	80.36**	72.48**
"F" blocks	....	....	1.95	2.91	3.23	3.54	....	....	....
S.D.	12	8	11	0.22	31.52	22.85	13.83	0.052	0.22

† Means based on 38 seedlings for drouth resistance and 36 for all others.
* Significant.
** Highly significant.

that the seedlings were in the drouth machine before they died; column three, the average number of hours between the first sign of injury and of death. The analysis of variance for time to injury and to death showed a highly significant difference between nitrogen treatments. The results are graphically shown in graphs G and H, figure 14. The seedlings receiving 170, 325, and 340 p.p.m. of nitrogen stood up in the machine as well as or better than the seedlings receiving no nitrogen. These results are in agreement with the findings of Wilde *(29, 30)*. He found that the seedlings receiving a well-balanced fertilizer survived as well under field conditions as did the seedlings grown under certain

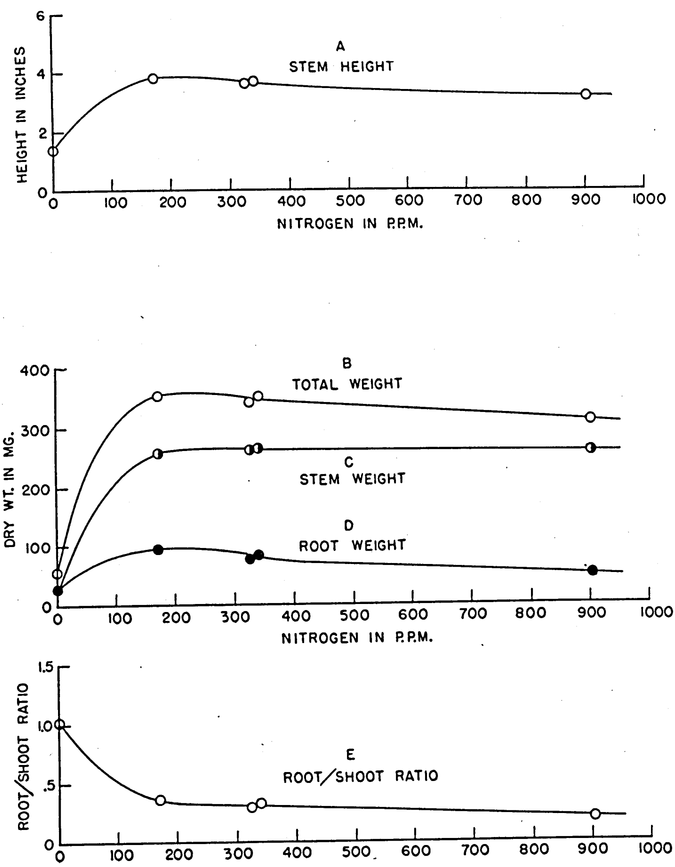
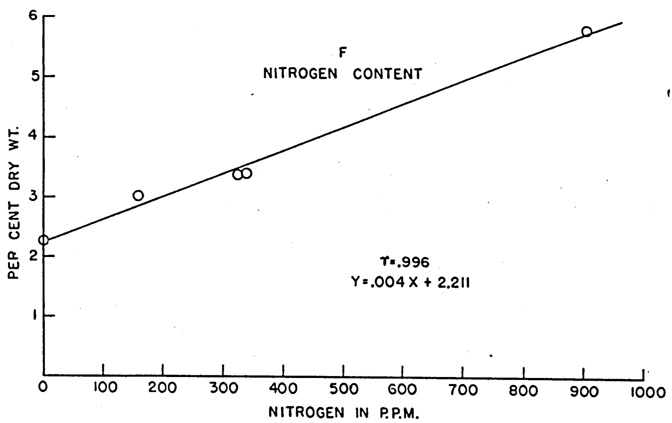


FIG. 13. Stem height, total weight, stem weight, root weight, and root/shoot ratio for seedlings grown in sand cultures with varied nitrogen supply

(Drouth experiment one)

deficiencies. Shirley and Meuli *(25)*, on the other hand, report quite different results. They used a drouth machine similar to the one used in this experiment. In their experiment nitrogen was added in the form of $(NH_4)_2SO_4$. There were three levels, 0, 50, and 100 p.p.m. The seedlings were grown in the same kind of jars as was used for the sand culture work reported in this bulletin but a fresh supply of culture solution was poured on the surface of the sand once a week. The nitrogen was added with this solution. If, for example, a jar received a weekly application of a solution containing 100 p.p.m. of nitrogen, it is probable that the total nitrogen supplied became exceedingly high toward the



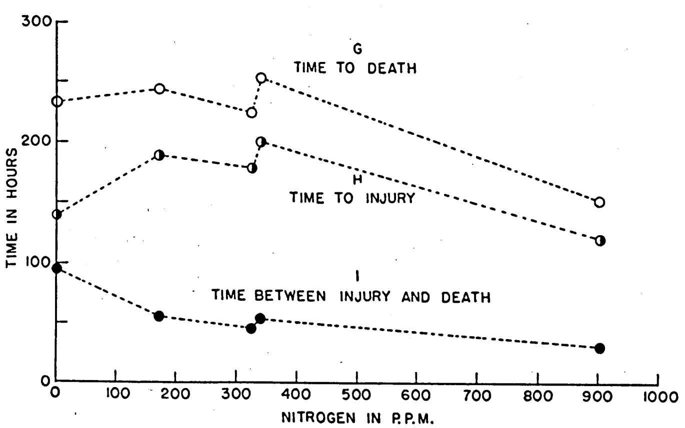


FIG. 14. Per cent of nitrogen in stems and drouth resistance (time to death, time to injury, time between injury and death), for seedlings grown in sand cultures with varied nitrogen supply
(Drouth experiment one)

end of the experiment. In fact, these two levels of nitrogen may be well over the optimum nitrogen concentration for jack pine seedlings and as a result there was a decrease in drouth resistance. If this were the case it would agree very well with the results shown in graphs G and H, figure 14. Here the highest supply of nitrogen had a decreasing effect on drouth resistance.

The analysis of variance for the number of hours between injury and death showed only a significant difference between treatments (see table 12 and graph I, figure 14). This "time" seems to decrease with the increase in the amount of nitrogen supplied, the elapse of time between injury and death being much greater for the seedlings receiving no nitrogen. It was observed after the drouth resistance test was completed that the sand was still moist in the jars which had received 905 p.p.m., while all the moisture had been removed from the rest. This shows the effect of nitrogen supply on the absorption of moisture, since the seedlings receiving an overdose died before they had depleted the moisture supply.

Effect of nitrogen on growth. After the drouth test had been completed, the height of stem, weight of stem, weight of root, and nitrogen content of stem were determined. The results of this analysis are given in table 12. Nitrogen treatments proved highly significant in every case, and blocks not significant. Stem height, total seedling weight, stem weight, root weight, and root/shoot ratio followed the same trend as they did in the first sand culture experiment, except that in the case of total and stem weight there was not such an abrupt drop in the curve around 300 p.p.m. In fact, after the peak was reached at 170 p.p.m. there seems to be a rather gradual decrease. This may be a result of the difference in the method of adding nitrogen. It will be remembered that in the first sand culture experiment the nitrogen was all added during the first few weeks of the experiment, while in this case it was added throughout the course of the experiment. The striking difference between this experiment and the 1939 sand culture experiment is in the percentage of nitrogen in the stems. In this last experiment there is a straight line relationship between the nitrogen supplied and the percentage in the seedlings (dry weight basis), while in the previous experiment the trend was curvilinear. Then, too, the nitrogen percentage in the stems was much greater for the 1940 experiment. Living in the drouth machine for 200 to 250 hours may have had a serious effect upon the nitrogen content of the seedlings especially when expressed on a percentage basis. The conditions in this machine were very favorable for respiration (owing to high temperature) but not so favorable for photosynthesis. This condition in combination with the scanty supply of moisture probably resulted in the burning by respiration of a considerable amount of stored food. Since respiration consumes carbohydrates first and proteins last, it is conceivable that the nitrogen content expressed in per cent might be considerably increased by such a treatment. Although this may account for the higher nitrogen percentage in the 1940 experiment, it can hardly be taken as an explanation of the difference in trend for the two experiments.

Once each week throughout the experiments, height measurements were made on 10 seedlings randomly marked at the beginning of the experiment. The seedlings receiving no nitrogen grew only slightly during the entire period. Those that received 170, 325, and 340 p.p.m. grew in height about one-half inch each week, while the height growth of those receiving 905 p.p.m. began to taper off after the eighth week. The final heights are shown in table 12.

DROUTH EXPERIMENT NUMBER TWO, FALL, 1940

Experimental Method

Apparatus, sand, culture solution, and seedlings. In the second drouth resistance experiment the same sand culture apparatus was again used. The same kind of sand was also used, but the method of starting the seedlings was somewhat different. After the sand had been placed in the jars, 3½ liters of distilled water was placed in each supply bottle. Then on August 16, 75 jack pine seeds of the 4.5 mg. class were planted in each jar. Then the jars were flushed, allowed to drain, and the upper level of the distilled water in the supply bottles marked. In this manner the seedlings were germinated right in the jars avoiding the necessity of transplanting from germination flats to the jars.

On September 4, when germination was nearly completed, the standard culture solution was placed in the supply bottles. This solution was mixed in such a manner that ½ liter placed in each bottle would bring the concentration of a 3½ liter volume in each system up to the proper level. Then as in the first drouth experiment, ½ liter of distilled water was added to each, making a total volume of 4½ liters in each system. When evaporation from the sand and transpiration from the seedlings reduced the volume of solution to 3½ liters (as indicated by the mark on the bottles), the solution had the same concentration of nutrient elements as given in table 1. The second addition of solution, which again consisted of an increase of one half the original concentration, was made October 15.

On September 9 germination was completed so the seedlings were thinned to 34 in each jar. Care was taken to select seedlings evenly spaced and as nearly as possible the same size. On September 14 they were thinned down to 30 in each jar, and on October 12, to 26. These thinnings were necessary in order to avoid overcrowding and even 26 in each jar was a few too many. This method of starting seedlings has both advantages and disadvantages. In the first place, it avoids the danger of injury to seedlings during the process of transplanting, and in the second place, no replacements are necessary since very little mortality occurs once the seedlings become established. The disadvantage is that although you have the same number in every jar at any given time, you do not have the same number in a jar throughout the experiment. The seedlings probably should have been thinned down to the final number before the nutrient solution was added. In this case most of the thinning was made within the first 10 days, but even then if one wishes to compare the results of this experiment with others in which a smaller number of seedlings had been grown in each jar, the nitrogen supplied should be reduced to a seedling basis.

Addition of nitrogen. The design of this experiment was a randomized block with 10 levels of nitrogen and two replications. The levels of nitrogen were 50, 100, 150, 175, 200, 225, 275, 325, 400, and 500 p.p.m. This nitrogen was supplied as in all the experiments in the form of NH_4NO_3. The fine growth of the seedlings in the first drouth resistance experiment pointed out the advantage of adding nitrogen in small amounts at frequent intervals throughout the course of the experiment. In this second drouth resistance experiment this procedure was followed. At the beginning of the experiment (September 4) all of the supply bottles were given enough nitrogen stock solution to give them a concentration of 25 p.p.m. Every addition afterwards increased the concentration of the solution in the system (solution in sand and supply bottle) by 25 p.p.m. These additions were made at equal intervals throughout the experiment. For example, the jars receiving 50 p.p.m. received 25 p.p.m. at the beginning of the experiment and 25 p.p.m. at the middle of the experiment (in this case on the 40th day). Likewise, the jar receiving 100 p.p.m. received four applications evenly spaced throughout the experiment, and so forth on to the jar receiving 500 p.p.m. which received

20 applications of 25 p.p.m. each evenly spaced over the 83 days of the experiment. This proved to be by far the best method of adding nitrogen, although it makes the results of the first sand culture experiment not quite comparable to the results of this study. The levels of nitrogen were spaced at close intervals to study in detail the effect of nitrogen supply on drouth resistance and growth over the practical and critical range of that supply.

Weather records. Evaporation and relative humidity were measured by Livingston atmometers and a hygrograph, respectively (the same as in previous experiments). These results are summarized in table 13. The daily minimum and maximum temperatures were also recorded. Weekly averages of these are presented in table 13. In the early fall, growth conditions were very good. There was a relatively high temperature and a fair amount of light, but later in the fall the days became much too short for maximum growth. In order to offset this effect upon seedling growth as much as possible, the day was lengthened by the use of artificial light. Five 200-watt electric lights were placed 28 inches above the surface of the sand. The bowl reflectors used to hold the lights were arranged so that an equal amount of light hit each jar. This distribution of light was checked by a Weston photoelectric cell.

Results and Conclusions

Effect of nitrogen on drouth resistance. On November 23 the jars were flushed for the last time and the supply bottles were disconnected from the jars. As was the case in every experiment some of the seedlings in the highest levels of nitrogen started to die toward the end of the experiment. For example, in this study there were two seedlings in the jar that had received 400 p.p.m. in replication one, and one dying in the same level in replication two. On November 25 before the jars were placed in the drouth machine these dead seedlings were removed, and enough other seedlings were randomly removed from the other jars to make a total of 22 healthy plants in each.

The test in the drouth machine was conducted in the same manner as that for the first drouth resistance experiment. The number of hours that the seedlings could live in the drouth machine was taken as a measure of their drouth resistance. The temperature in this case was set at 95° F. and the wet bulb thermoregulator at 69° F. This resulted in a relative humidity of approximately 26 per cent. This lower humidity was made possible by using a better thermoregulator for the wet bulb. In the

Table 13. Temperature, Relative Humidity, and Atmometer Records, Forestry Greenhouse, 1940

Date	Temperature, weekly averages		Relative humidity, weekly averages		Evaporation in c.c. for atmometers					
					Set number 1			Set number 2		
	Maximum	Minimum	Day	Night	Black	White	Difference	Black	White	Difference
September 5-8	96	68	56	83	173	144	29	187	145	42
September 9-15	89	52	41	76	326	286	40	385	285	100
September 16-22	93	67	58	81	296	266	30	358	266	92
September 23-29	87	57	44	68	419	370	49	452	353	99
September 30-October 6	91	69	58	69	282	246	36	277	225	52
October 7-13	91	70	51	67	341	306	35	356	299	57
October 14-20	86	68	47	64	380	318	62	406	332	74
October 21-27	86	67	53	69	329	285	44	339	292	47
October 28-November 3	84	68	62	70	270	241	29	289	243	46
November 4-10	83	69	68	68	267	249	18	266	254	12
November 11-17	81	70	52	51	359	318	41	367	332	35
November 18-23	85	74	58	55	267	252	15	243	229	14
Totals	......	......	......	......	3,709	3,281	428	3,925	3,255	670

Total black	7,634 c.c.
Total white	6,536 c.c.
Difference	1,098 c.c.

first drouth experiment an attempt was made to get a relative humidity of around 25 per cent but because of the faulty wet bulb thermoregulator this was not achieved. At the beginning of this second drouth test there was a variation of as much as 5 per cent in the relative humidity, but after the moisture had been fairly well depleted from the jars the variation was reduced considerably. On the morning of November 25 the jars containing 22 seedlings each were randomly arranged on the revolving table in the drouth machine. Before they were put in, however, the photograph shown in figure 15 was taken. This picture testifies to the fine growth and vigor of these seedlings. It was found in the first drouth experiment that it was not necessary to take mortality readings every two or four hours but that about three such readings a day were sufficient. These counts were, therefore, made at 7 a.m., 3 p.m., and 11 p.m. each day. The last count was taken 419 hours after the drouth test was started.

The results of this drouth resistance test are summarized in table 14. The analysis of variance showed highly significant differences between nitrogen supply and both the number of hours to injury and to death. But, in the case of the elapse of time between injury and death the analysis indicated that no such difference occurred. These results are in agreement with the first drouth experiment, for in that case it was only the seedlings receiving no nitrogen that continued to live longer after injury than the rest. The trends of these three measurements are presented in graphs G, H, and I, figure 16. The seedlings receiving 200 to 300 p.p.m. of nitrogen again lived as long in the machine as seedlings receiving the least amount of nitrogen and longer

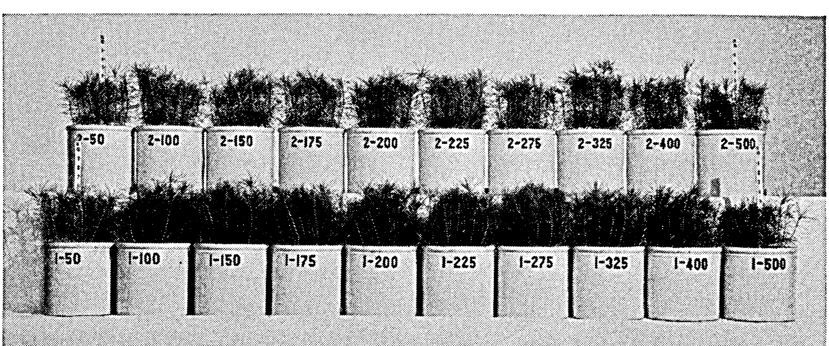


FIG. 15. Second sand culture experiment, fall, 1940
The first number on the jars refers to the replication and the second to the nitrogen supplied in p.p.m.

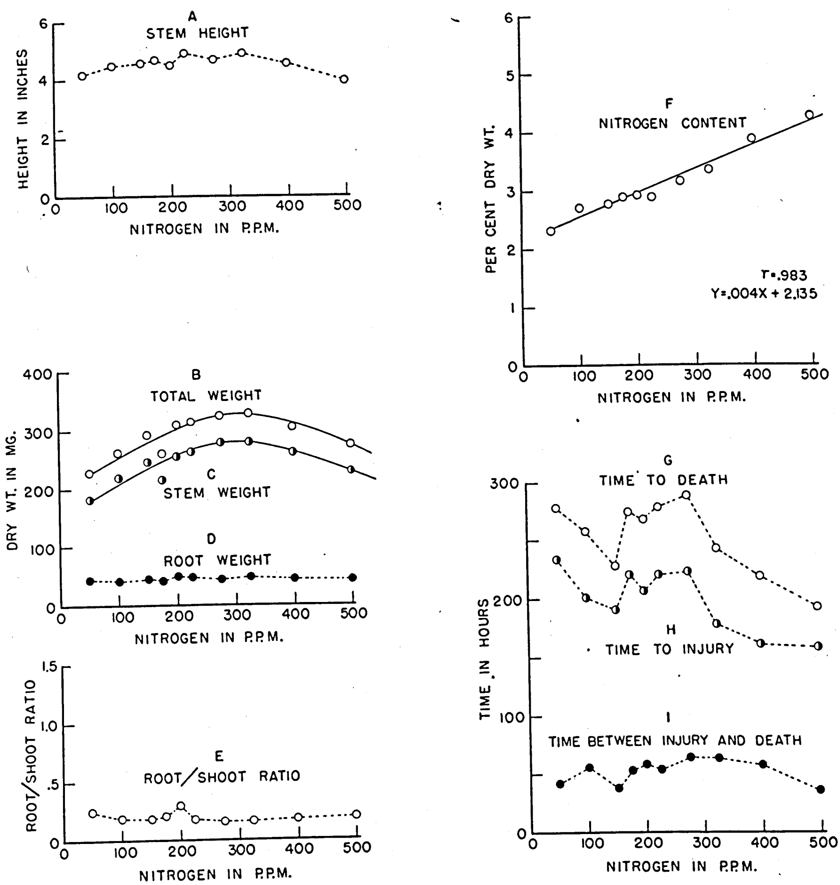


FIG. 16. Stem height, total weight, stem weight, root weight, root/shoot ratio, per cent of nitrogen in stems, and drouth resistance (time to death, time to injury, time between injury and death), for seedlings grown in sand cultures with varied nitrogen supply

(Drouth experiment two)

than seedlings receiving an oversupply. As was found in the previous experiment, the sand in the jars receiving the highest level of nitrogen (500 p.p.m.) was moist after all the seedlings had died. These results emphasize the fact, previously stated, that if jack pine seedlings receive a well-balanced nutrient supply (nitrogen in correct proportion with other elements), they will be at least as drouth resistant as seedlings deficient in nitrogen. This again discredits the old belief that increase in nitrogen produces less drouth resistance. If only the first three levels of nitrogen are considered, there is a decrease in drouth resistance

Table 14. Summary of Treatment Means† and Analysis of Variance—Drouth Experiment Number Two, Fall, 1940

Nitrogen treatment	Drouth resistance			Stem height	Mean weight			Root/shoot ratio	Nitrogen in stem
	Time to injury	Time to death	Time between injury and death		Total	Stem	Root		
	Hours	Hours	Hours	Inches	Mg.	Mg.	Mg.		Per cent
50	235	278	43	4.15	226.5	181.5	45.0	0.246	2.33
100	202	259	57	4.47	261.2	219.7	42.4	0.194	2.72
150	190	229	38	4.55	291.5	247.0	44.6	0.180	2.79
175	221	275	53	4.60	258.2	215.4	42.8	0.204	2.90
200	208	267	59	4.47	306.0	255.6	50.4	0.198	2.93
225	221	278	54	4.87	312.2	262.6	49.6	0.188	2.90
275	223	288	65	4.63	322.8	278.0	44.8	0.162	3.17
325	179	442	64	4.86	325.8	278.7	47.0	0.168	3.36
400	161	219	58	4.47	303.6	259.4	44.2	0.170	3.88
500	158	193	36	3.84	272.4	228.6	43.7	0.193	4.29
"F" treatments	8.01**	9.71**	2.74	2.58	6.35**	6.07**	....	1.04	178.77**
"F" blocks	3.49	....	2.86	....	1.58	....	....	....	....
S.D.	13	14	9	0.27	18.2	17.8	6.18	0.033	0.06

† Means based on 22 seedlings for drouth resistance and 26 for all others.
** Highly significant.

with increase in nitrogen supply, but at the next higher levels the reverse trend is true. Only further experimentation could determine whether or not this dip is a real difference or purely a sampling error. It nevertheless hints at the importance of nitrogen balance to drouth resistance.

Effect of nitrogen on growth. In table 14 will be found the summary of the growth measurements and nitrogen content of the seedlings at the end of the drouth resistance run. The analysis of variance indicated a highly significant treatment difference in the case of total weight, stem weight, and nitrogen percentage, but the height growth and root/shoot ratio showed no such differences. There was a definite trend (graph A, figure 16) in the relation of height to nitrogen supply but the difference between nitrogen levels was not great enough to be detected by the analysis of variance. The root weight and root/shoot ratio remained constant for all levels of nitrogen (graphs D and E, figure 16). This is to be expected since the optimum level of nitrogen for root growth is between 50 and 100 p.p.m. The best growth in terms of dry weight was attained at a nitrogen concentration of about 300 p.p.m. (graphs B and C, figure 16). The reason for this supply in p.p.m. being greater than the results found by previous experiments is no doubt related to the number of seedlings grown in the pots, the drouth resistance run itself, or the method of adding the nitrogen to be discussed later.

The nitrogen content of the stem expressed in terms of dry weight is shown in both table 14 and graph F in figure 16. A straight line was mathematically fitted to the points. The correlation coefficient (8 degrees of freedom) and the formula are given below the graph. This straight line relationship between nitrogen supply and nitrogen content is almost identical with that found in the first drouth experiment (see graph F, figure 14). In plotting the lines in both cases the slopes were carried out to six places of decimals but are presented abridged on the graph.

One other point that should be mentioned in connection with the effect of nitrogen supply upon growth is seedling color. As in previous experiments, in the highest level of nitrogen a few of the seedlings turned a light yellow color.

DISCUSSION

If the results of the sand culture experiment (table 4), drouth experiment one (table 12), and drouth experiment two (table 14) are compared, it will appear at first that there is not very close agreement as to the optimum concentration of nitrogen. In the sand culture study the best growth was attained at a nitrogen supply of 230 p.p.m. At this level of nitrogen the seedlings reached the average height of 4.82 inches and average weight of 909.28 mg. On the other hand, in drouth experiment two the seedlings attained an average height of 4.87 inches at 225 p.p.m. of nitrogen and 4.86 inches at 325 p.p.m. The original data showed the greatest height for block 1 at 275 p.p.m. and for block 2 an equal height for 225 and 325 p.p.m. This lack of agreement between blocks is probably the reason the "F" value in the analysis did not reach the 5 per cent level. The greatest average seedling weight occurred in the jars receiving 325 p.p.m.; but even though the seedlings in that level of nitrogen were as tall as those in the optimum level in the sand culture experiment, they weighed on the average only 325.8 mg. This small weight in relation to height is evidence again of the effect of the drouth test itself upon seedling weight. This point has been previously discussed, but its particular bearing on this experiment should be mentioned here. The average seedling weight was 322.8 mg. at 275 p.p.m. and 225.8 mg. at 325 p.p.m. If one checks the drouth resistance figures in table 14 for those two levels of nitrogen it will be found that at 275 p.p.m. the seedlings lived 46 hours longer than at 325 p.p.m. The fact that in the sand culture experiment, seedlings 4.82 inches in height weighed 909 mg., while at the end of drouth experiment two, seedlings of almost the same height weighed only 325 mg., indicates that it is probable that respiration during the drouth test reduces weight very considerably. Now since the seedlings in drouth experiment two receiving 325 p.p.m. were in the machine 40 hours less than those receiving 275 p.p.m., it is reasonable to assume that if they had been weighed before the test, the seedlings which had received 275 p.p.m. would have weighed the most. This can only be proved by an experiment designed to detect that difference. But for all practical purposes, considering both drouth resistance and seedling weight, it can safely be said that 225 to 275 p.p.m. gave the best growth.

If we wish to make a more detailed comparison of the three experiments under consideration, the nitrogen supply for the optimum growth in each case must be reduced to a seedling basis. This is necessary since there was a different number of seedlings grown in the jars in the various experiments. In the sand culture experiment there were 25 seedlings in each jar, and in drouth experiment one, 22 in each jar. In drouth experiment two, however, there were 34 seedlings in each jar for 11 days, 30 for the next 28 days, and 26 for the remaining 44 days of the experiment. This made an average for the whole experiment of a little more than 28 seedlings in each jar. Now, we can compute the nitrogen supply for optimum growth on a seedling basis in each experiment as follows:

Sand culture experiment	230 p.p.m. ÷ 25 = 9.20 p.p.m.
Drouth experiment one	175 p.p.m. ÷ 22 = 7.95 p.p.m.
Drouth experiment two	225 p.p.m. ÷ 28 = 8.03 p.p.m.
and	275 p.p.m. ÷ 28 = 9.82 p.p.m.

Two levels were used for the last experiment because the seedlings grew very nearly as well in both. The results can now be changed back to a standard of 25 seedlings in each jar as was used in the sand culture experiment.

Sand culture experiment	9.20 p.p.m. × 25 = 230 p.p.m.
Drouth experiment one	7.95 p.p.m. × 25 = 198 p.p.m.
Drouth experiment two	8.03 p.p.m. × 25 = 200 p.p.m.
and	9.82 p.p.m. × 25 = 245 p.p.m.

The true relationships between the experiments can now be seen. The nitrogen supply for optimum growth was within a range of 50 p.p.m for all the experiments. This is as close an agreement as can be hoped for, using the experimental technique employed in these investigations. Not much weight can be given to the comparison in the case of drouth experiment one because so few levels of nitrogen were used. The real optimum level could well have fallen between 170 and 325 p.p.m.

In making the comparison just discussed it was necessary to assume (in order to obtain the average number of seedlings in drouth experiment two) that a seedling regardless of age will absorb nitrogen at the same rate. Although this assumption may seem wrong at first, there is evidence that it is accurate enough for this purpose. In the sand culture experiment the nutrient solution was analyzed for nitrogen at the middle and end of the experiment. It was found that the rate of depletion, although greater for older seedlings, would not cause a serious error in computing such an average.

In making comparisons between experiments, the effect of the method of adding nitrogen on seedling growth should not be overlooked. If, for example, a jar to be supplied with 500 p.p.m. receives that amount in a few weeks at the beginning of the experiment, as was the case in the sand culture experiment, the results may be quite different than if the supply had been added gradually over the whole study as in the second drouth experiment. In the first case the nitrogen concentration in the nutrient solution would be built up to a peak in the first part of the experiment and then gradually depleted by the seedlings to the termination of the investigation. This means that the small seedlings have the most nitrogen available and when they get older there is less. But if the nitrogen is added at equal intervals all through the experiment, the concentration of the element is more constant. If the nitrogen is completely exhausted from the solution between applications, as would be the case in a low level, the concentration would be raised to its original level with each application, thus holding it rather constant. If the nitrogen in the nutrient solution was not completely exhausted between applications, then there would be a gradual building up of the concentration to the end of the experiment. This seems to be the most desirable situation, since older seedlings can use more nitrogen than younger ones.

Application of Nitrogen in the Nursery

When one has demonstrated the beneficial effects of supplying growing seedlings with an optimum amount of nitrogen and has found a nursery soil to be deficient in the element, the next step is the actual application of the fertilizer to the seedbeds. The method of application and the amount to apply remain the next problem. With this idea in mind, a nursery field experiment was designed to determine if possible the amount of nitrogen required for optimum growth of both one- and two-year-old jack pine planting stock. The study was made in the nursery of the University Forest Experiment Station, Cloquet, Minnesota.

EXPERIMENTAL METHOD

Field design. The nursery field experiment was set up in the form of a 4 x 4 latin square with four levels of nitrogen (10, 50, 100, 200 p.p.m.) and four replications. Each plot consisted of a bottomless wooden box 14 inches deep, 16 inches wide, and 20 inches long. After these boxes had been waterproofed with a mixture of paraffin wax and gasoline they were set down in the soil so that about three inches of the upper edge stood above the surface of the soil. The soil in the boxes was screened and replaced in such a manner that the surface soil remained at the surface and was not mixed with the soil at the bottom. This screening was necessary to facilitate removal of the seedlings at the end of the experiment. Figure 17 shows the arrangement of the boxes.

FIG. 17. Nursery field experiment

Addition of fertilizer. A complete liquid fertilizer, with variations in the amount of nitrogen, was supplied to the boxes. A solution containing 139.00 mg. per liter of KH_2PO_4, 222.50 mg. of $MgSO_4.7H_2O$, 166.88 mg. of $CaCl_2.2H_2O$, and 2.50 mg. of iron citrate was prepared. Then to 4 liters of this solution enough NH_4NO_3 (in form of stock solution) was added to give that volume of solution a nitrogen concentration of 10 p.p.m. One liter of this solution was then sprinkled evenly over each of the four boxes of that nitrogen level. After this another 4 liters were drawn from the original solution and enough nitrogen added to result in a concentration of 50 p.p.m. One liter of this solution was given to the boxes so labeled. In a similar manner the nitrogen for the 100 and 200 p.p.m. levels was added.

During the first summer the boxes received six such applications of fertilizer with an interval of two weeks in between them, starting July 5 and ending September 13, 1939. This was found to be insufficient, so the second summer they were given an application every week, making twice the number of fertility treatments during the 1940 growing season. As will be shown later, this was sufficient for 1-0 seedlings but not for two-year-old stock.

Planting seeds and care of seedlings. On June 13, 1939, before any fertilizer had been added, 600 jack pine seeds were planted in each box. The seeds were planted in three rows running lengthwise of the box. On July 5 after germination had been completed, the plots were given their first fertility treatment. At the end of the growing season there were between 200 and 300 seedlings in each box.

On June 3, 1940, two rows of seedlings from each box were carefully lifted and replanted. Before they were replanted, all the small or cull seedlings were sorted out. Then a row of 15 large seedlings was transplanted back into the box from which the seedlings came, next one row of small or cull seedlings, and finally the row that remained was thinned so that there were 15 seedlings in each of the three rows in each of the 16 boxes. In addition to that a row of 200 seeds was planted in each box. This made it possible to have, at the end of the second year, the following kinds of planting stock:

1-0 seedlings	Large 1-1 transplants
2-0 seedlings	Small 1-1 transplants

Each box contained a random arrangement of these four sizes of stock, making the experimental design a split plot latin square. The transplanting was completed June 5, and on June 11, 1940, the boxes received their first fertility treatment of that season. On July 15 after the germination of the seeds was completed, the young seedlings were thinned to 50 in each row. By August 1, they had become crowded so were thinned to 22 in each row.

During the experiment there were only three plants of the two-year-old stock that died. The mortality in the one-year stock was likewise very low. The mortality was held at such a low level by treatment of the soil to prevent damping off during the germination period and by placing screens over the growing seedlings to protect them from insects and rodents.

Weather records. The first summer (1939) of the experiment was the same summer during which the pot culture test of Cloquet nursery soil was made and consequently the weather records reported in part two of this paper will not be restated here. During the second growing season no weather records were taken. The rainfall records for this experiment are not of much value because whenever the soil became dry the plots were sprinkled.

RESULTS AND CONCLUSIONS

Effect of nitrogen supply on seedling growth. At the end of the first year a sample of 25 seedlings (stems only) was randomly taken from each box. The average mean weights for the four levels of nitrogen were: 10 p.p.m.—40.09 mg.; 50 p.p.m.—44.90 mg.; 100 p.p.m.—53.90 mg.; 200 p.p.m.—60.50 mg. The analysis of variance showed a significant difference between nitrogen treatments and also between columns. The standard deviation was 6.41 mg. About the only practical conclusion one can draw from these results is that there appears to be an increase in seedling weight with an increase in nitrogen supplied and since 200 p.p.m. resulted in the largest seedlings it is likely that the optimum level was not reached. It was for this reason that the treatment was doubled the second season.

During the last two weeks in September at the end of the second season (1940) the seedlings and transplants were carefully removed from the boxes. Deep trenches were dug alongside of the plots and after one side of the box was removed, the soil was washed away from the roots, using a small stream of water. A representative seedling taken from each size of stock and each level of nitrogen is shown in figure 18. Ten seedlings were then randomly selected for analysis from each of the four sizes of stock in each of the 16 boxes. The means of stem height, stem diameter, total weight, stem weight, root weight, and root/shoot

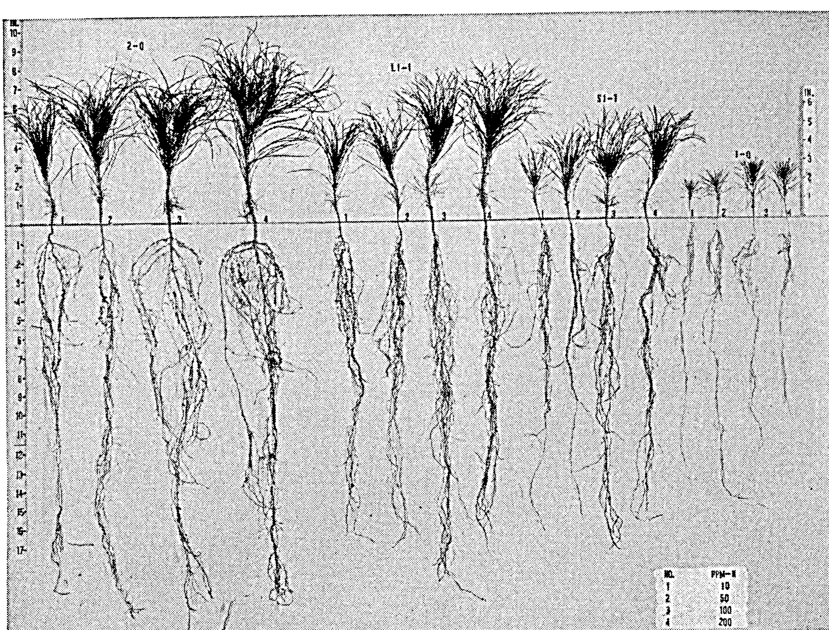


FIG. 18. 2-0 seedlings, large 1-1 transplants, small 1-1 transplants, and 1-0 seedlings grown in the nursery with varied nitrogen supply
(Nursery field experiment)

ratio for all four replications are given in table 15. There was such a difference between the weights of 1-0 seedlings and the two-year-old seedlings and transplants that a split plot analysis was impossible, since in such an analysis you assume that the standard deviation of each of the sizes of stock is approximately the same. This same difficulty was true for everything except root/shoot ratio, and as a result a latin square analysis of variance was individually run for each size of stock. The results of these analyses are summarized in table 15.

Table 15. Summary of Treatment Means† and Analysis of Variance—Nursery Field Experiment, Summers of 1939 and 1940

Nitrogen treatment	Stem height	Stem diameter	Mean weight			Root/shoot ratio
			Total	Stem	Root	
	Inches	Mm.	Mg.	Mg.	Mg.	
			1-0 SEEDLINGS			
10	1.19	0.66	57.26	30.53	26.73	0.880
50	1.50	0.78	87.69	52.56	35.14	0.667
100	1.97	0.88	99.15	67.78	31.36	0.463
200	2.01	0.87	91.47	64.87	26.60	0.408
"F" columns	....	1.00	1.43	1.10	1.58	3.03
"F" rows	....	....	....	....	....	....
"F" treatment	20.76**	16.78**	10.05**	23.81**	1.61	31.63**
S.D.	0.17	0.05	11.60	6.94	6.47	0.076
			2-0 SEEDLINGS			
10	3.42	2.11	1,001.23	633.22	368.01	0.587
50	4.52	2.79	1,812.26	1,234.59	577.67	0.468
100	5.30	3.36	2,552.43	1,831.10	721.34	0.398
200	5.74	3.46	2,816.22	2,030.79	785.43	0.390
"F" columns	7.54*	3.48	....	....	1.62	2.22
"F" rows	....	7.58*	5.47*	3.28	7.11*	1.87
"F" treatment	27.05**	158.19**	104.70**	79.50**	34.82**	9.11*
S.D.	0.39	0.10	159.42	141.56	62.72	0.060
			LARGE 1-1 TRANSPLANTS			
10	3.20	1.67	569.35	336.61	232.74	0.694
50	3.48	2.04	876.02	556.49	319.53	0.580
100	4.02	2.48	1,302.71	876.48	426.23	0.498
200	4.41	2.65	1,561.69	1,059.75	501.94	0.476
"F" columns	3.60	2.49	1.40	1.23	1.40	....
"F" rows	....	....	....	....	....	....
"F" treatment	8.96*	16.47**	18.49**	16.65**	15.08**	5.65*
S.D.	0.36	0.22	205.23	158.33	60.90	0.083
			SMALL 1-1 TRANSPLANTS			
10	2.01	1.26	287.60	166.32	121.28	0.726
50	2.08	1.52	419.20	265.70	153.50	0.574
100	2.59	1.86	648.11	437.27	210.84	0.466
200	3.00	2.04	801.98	556.48	245.51	0.441
"F" columns	3.53	1.12	....	....	....	....
"F" rows	....	....	....	....	....	1.37
"F" treatment	6.21*	7.89*	4.89*	6.99*	2.08	15.09**
S.D.	0.38	0.25	208.03	131.67	77.47	0.067

† Means based on 40 seedlings each.
* Significant.
** Highly significant.

The data are also presented for each size of stock in graphs A, B, C, and D, figures 19 and 20. The height and diameter of the stems followed the same trend as weight and are, therefore, not shown in the figure. The first thing apparent is that the optimum nitrogen supply for the growth of 1-0 seedlings was reached (100 p.p.m.). This was not true for the two-year-old stock, for in all three sizes of this older stock the best growth was obtained at the highest level of nitrogen (200 p.p.m.). This was unfortunate, but the results demonstrate the beneficial effect of additions of nitrogen and present a steppingstone for further investigation. The root/shoot ratio in every case decreased with increase in nitrogen supply up to 100 p.p.m. beyond which it remained rather

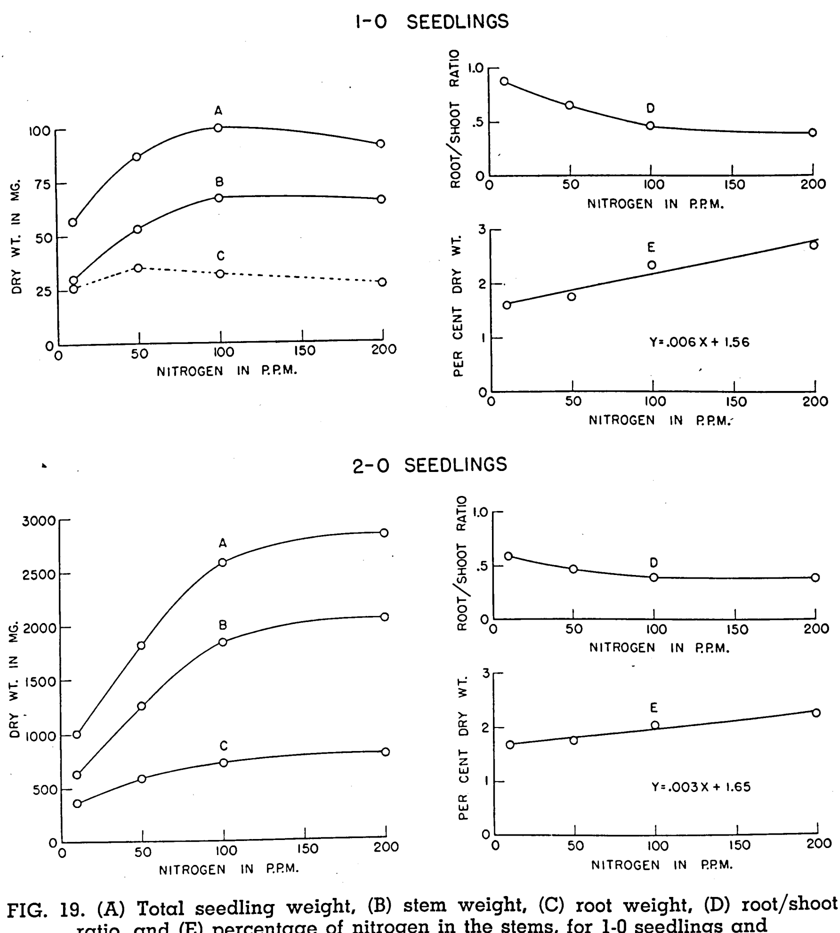


FIG. 19. (A) Total seedling weight, (B) stem weight, (C) root weight, (D) root/shoot ratio, and (E) percentage of nitrogen in the stems, for 1-0 seedlings and 2-0 seedlings grown in the nursery with varied nitrogen supply

constant. In figures 19 and 20 curves were fitted to the points for all measurements that showed a significant treatment difference. The rest are connected by dotted lines.

The two-year-old seedlings grew much larger than the transplants but the root/shoot ratio was not as high (see graphs A, B, C, and D under 2-0 seedlings and large 1-1 transplants, figures 19 and 20). Just what the survival of these two sizes of stock would be after planting in the field would have to be determined experimentally. Not much weight can be given to the results of the small 1-1 transplants because they were the smallest seedlings in each box transplanted back into the box. Their size at the time of transplanting was not uniform for all of the boxes but they do

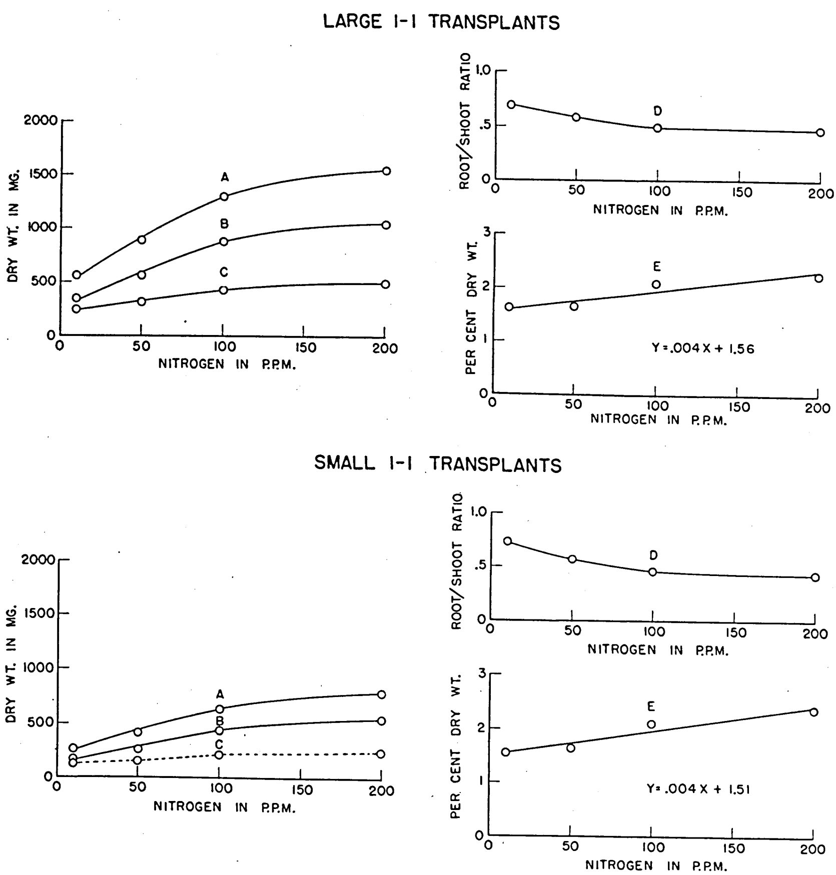


FIG. 20. (A) Total seedling weight, (B) stem weight, (C) root weight, (D) root/shoot ratio, and (E) percentage of nitrogen in the stems, for large 1-1 transplants and small 1-1 transplants grown in the nursery with varied nitrogen supply

represent the seedlings that would be normally culled at the time of transplanting. The results show that if these seedlings are properly fertilized they will develop into fair planting stock. The small 1-1 transplants receiving 200 p.p.m. of nitrogen caught up to the large 1-1 stock receiving 10 p.p.m. This suggests the possibility of eliminating a considerable portion of the culls by proper fertilization. This suggestion was further verified by the fact that at the end of the first season the percentage of small seedlings was much less in the plots receiving the highest supply of nitrogen.

Effect of nitrogen supply on nitrogen content. The analysis of nitrogen in the seedlings presented further interesting data concerning the relation of the supply of that element and the percentage in the seedlings. Table 16 gives the mean percentages for the four replications. The statistical analysis was run as a split plot latin square, the results of which are shown at the base of the table. An increase in nitrogen percentage with increase in supply is the first result apparent. Secondly, 1-0 seedlings have a higher nitrogen percentage in relation to nitrogen supplied than the older stock. The interaction between size of stock and nitrogen supply was highly significant but is not of much practical value.

Straight lines were fitted by formulae to the data for each size of stock (see graph E in each size class, figures 19 and 20). Here again both the formulae and lines show the close similarity of the older seedlings and the greater nitrogen content of the 1-0 stock. There is some doubt as to the correctness of fitting straight lines to these data, but for practical purposes it works very well. If we compare the pot culture experiment with this field experiment we will find that in the former the best growth was obtained

Table 16. Summary of Mean Nitrogen Content† and Analysis of Variance‡—Nursery Field Experiment, Summers of 1939 and 1940

Nitrogen treatment	Size of stock			
	1-0	2-0	Large 1-1	Small 1-1
10	1.60	1.69	1.61	1.56
50	1.76	1.75	1.63	1.63
100	2.34	2.05	2.09	2.12
200	2.70	2.27	2.25	2.36

† Nitrogen content in seedlings is expressed on basis of per cent dry weight. Means in body of table based on analysis of a sample of 10 seedlings from each of four replications.

‡ Analysis of variance made by split plot method.

"F" value for nitrogen treatments	111.38**
Standard deviation	.14
"F" value for size of stock	18.56**
"F" value for interaction between size of stock and nitrogen treatment	5.96**
Standard deviation	.09

** Highly significant.

from a nitrogen supply which gave a nitrogen content in the seedlings of 2.17 per cent, while in the latter the best growth was obtained at a supply which resulted in an internal nitrogen content of 2.16 per cent (determined by formula, graph E, 1-0 seedlings, figure 19). Now referring back to the sand culture experiment, the supply of nitrogen that gave the best growth again resulted in an internal seedling content of 2.23 per cent, that is, if we take the highest point of the curve (graph G, figure 7) and determine the nitrogen percentage from the formula given in figure 11. These comparisons partially summarize the objectives of the entire investigation. They point out that the following three objectives have been met:

1. The percentage of nitrogen in jack pine seedlings grown under optimum nitrogen balance has been determined by the sand culture experiment.
2. The approximate nitrogen deficiency of Cloquet nursery soil has been demonstrated by the pot culture test (see part two for discussion).
3. The amount of nitrogen that should be added in the nursery to give the best growth was determined by the nursery field experiment. It was known that the optimum nitrogen supply had been reached when the seedlings contained about 2.20 per cent.

Although these results refer to 1-0 seedlings, they could probably be applied to two-year-old planting stock as well. If one compares the nitrogen percentage for 1-0 seedlings, 2-0 seedlings, and 1-1 transplants, it will be apparent that when supplied with an optimum amount of nitrogen all the sizes of stock have an internal concentration of from 2.25 to 2.35 per cent. This is true even though the optimum supply was 100 p.p.m. for 1-0 seedlings and 200 p.p.m. for the older stock. It would seem, therefore, that a nitrogen percentage of 2.25 ± 0.10 indicates an optimum supply of that element both for one- and two-year-old stock.

DISCUSSION

The results of this experiment show that the best growth was obtained for 1-0 seedlings when they were supplied every week with 1 liter per box of a solution containing 100 p.p.m. of nitrogen. Changing this to a square foot basis we find that it is 0.45 liters per square foot. Since NH_4NO_3 contains 35 per cent nitrogen this would mean that this solution would contain 285.7 g. per liter of NH_4NO_3. The amount of nitrogen to give two-year-old stock was not so accurately determined, but the slopes of the curves indicate that the highest level was near the optimum. All that can be said is that the best growth of 2-0 seedlings and 1-1 transplants is around 200 p.p.m. or slightly over, that is when 0.45 liters per square foot is applied each week. When nitrogen is added in a soluble form, it is necessary to make frequent applications in order to compensate for its rapid leaching down beyond the reach of seedling roots. There are, therefore, two variables concerned, frequency of application and concentration of nitrogen in the solution applied. Only further experimentation can determine the inner relation of these two variables.

There are two common methods of applying this solution fertilizer. First, one might sprinkle it on, using hand sprinkling cans or power sprays. Second, it may be put through the overhead sprinkling system. There is some doubt as to the practicability of the latter method because of the danger of plugging up the system. It must be remembered that the results of the nursery soil experiment are applicable only to the Cloquet nursery, but they do demonstrate a successful method of adding nitrogen.

Summary of All Experiments

The experiments reported in this bulletin were devised to study the effect of nitrogen on the growth and drouth resistance of jack pine (*Pinus banksiana* Lamb.) seedlings and transplants. The results of the five experiments will be briefly summarized.

In the first experiment, a sand culture study, the seedlings were grown in one-gallon glazed earthenware jars supplied by irrigation from the bottom with a culture solution in which only nitrogen was varied. The experiment terminated at the end of 83 days. The seedling shoots grew largest in the jars receiving 200 p.p.m. of nitrogen, decreasing in weight and height on each side of this point. The roots, however, reached approximately their greatest weight at 100 p.p.m., with little change with further increase in nitrogen. The root/shoot ratio decreased rapidly until the nitrogen supply reached 100 p.p.m. After that there was little or no change. Nitrogen percentage of the seedlings expressed on a dry weight basis showed a steady increase with increase in the supply of nitrogen. The percentage in the roots was a little less than that in the stems. At the nitrogen supply that resulted in maximum growth the seedlings contained approximately 2.20 per cent of that element (average for root and stem for supply of 200 p.p.m.).

The second experiment was designed to determine by a pot culture test the amount of available nitrogen in the nursery soil at the University Forest Experiment Station, Cloquet, Minnesota. The experimental method was much the same as that used for the sand culture study except that instead of putting sterile soil in the jars, nursery soil was used. The seedlings growing in these jars were supplied with various levels of nitrogen. A total supply of 100 p.p.m. gave the best growth, and since the best growth in the sand culture experiment was obtained at a supply of 200 p.p.m., this indicated that the soil supplied the equivalent of 100 p.p.m. The nitrogen in the soil can also be determined by the use of the percentage of nitrogen in the seedlings. In the pot culture test of nursery soil the largest seedlings contained 2.17 per cent nitrogen. According to the results of the sand culture experiment, seedlings containing that amount of nitrogen should have received 189 p.p.m. Since they actually received only 100 p.p.m., the soil must have supplied the equivalent of 89 p.p.m. These two methods of determining soil nitrogen check very closely and show that the nursery soil contains only about 40 to 50 per cent as much nitrogen as it should.

In the native habitat of jack pine it is not only necessary to produce well-developed stock but also to produce drouth-resistant stock as well. The third and fourth experiments were designed to determine the effect of nitrogen on drouth resistance. In both experiments, seedlings grown in sand cultures with varied nitrogen supply were subjected to artificial drouth. Drouth resistance was measured by the number of hours the seedlings could live in a machine especially constructed to simulate actual drouth conditions. It was found that the seedlings receiving the optimum nitrogen supply were the most drouth resistant. They stood up only slightly longer than seedlings very deficient in nitrogen, but the important thing is that they were at least as resistant to drouth as those seedlings. With addition of nitrogen over the optimum the resistance to drouth greatly decreased.

After the nitrogen requirements of jack pine seedlings had been determined and it had been demonstrated that seedlings growing under an optimum supply of that element were drouth resistant, the next step was the practical application of the results. The preliminary step in this application had already been taken, for in the pot culture study it was found that Cloquet nursery soil contains only about 40 to 50 per cent as much nitrogen as is required for best growth. The fifth experiment was designed to determine the amount of nitrogen that must be added to the nursery soil to make up for this deficiency. Plots, consisting of bottomless wooden boxes set down in the soil, were arranged in the nursery in the form of a 4 x 4 latin square with four levels of nitrogen and four replications. The fertility treatments consisted of applications of a liquid fertilizer at the rate of 0.45 liters per square foot. These applications were made every two weeks the first season and every week the second. In the first level of nitrogen this liquid fertilizer contained 10 p.p.m. of the element, in the second 50 p.p.m., and in the third and fourth 100 and 200 p.p.m., respectively. It was found that the 1-0 seedlings grew best when supplied every week with a fertilizer solution containing 100 p.p.m. of nitrogen, while 2-0 seedlings and 1-1 transplants, on the other hand, grew best at the highest level of nitrogen (200 p.p.m. supplied at the rate of 0.45 liters per square foot per week). Since the growth of the two-year-old stock increased to the highest level of nitrogen, the real optimum may not have been reached.

The nitrogen percentage of the three sizes of stock also showed some interesting relationships. In the 2-0 seedlings and 1-1 transplants growing under an optimum supply of 200 p.p.m. of nitrogen, the internal percentage of that element was 2.27 per cent and 2.25 per cent, respectively, while in 1-0 seedlings growing under an optimum supply of 100 p.p.m., the nitrogen percentage was 2.34 per cent. These results check very closely with the nitrogen percentage in seedlings grown with the optimum supply of nitrogen in the sand culture experiment. Apparently around 2.25 ± 0.20 per cent nitrogen in either one- or two-year-old stock indicates that they have been grown with an optimum supply of that element. This, of course, is true only when all other nutrients are adequate and other growing conditions are favorable.

The results of the entire investigation may be briefly summarized in the following conclusions:

1. The height and weight of the seedling stems increased with increase in the supply of nitrogen up to 200 to 250 p.p.m. Beyond this there was a decrease in both weight and height.

2. The weight of the roots increased up to 100 p.p.m. and further increase in nitrogen supply resulted in little change.

3. The root/shoot ratio decreased with increase in available nitrogen until a supply of 100 p.p.m. was reached, after which there was little or no change.

4. The percentage of nitrogen in the stems and roots increased with increase in the supply of that element. The percentage of nitrogen in the seedlings (average for roots and stems) for the

optimum nitrogen supply was approximately 2.20 per cent for both sand cultures and nursery soil.

5. The nursery soil at the Cloquet Forest Experiment Station contained about one half the amount of nitrogen required for best growth.

6. Seedlings grown under an optimum nitrogen supply (200 to 250 p.p.m.) were as drouth resistant as seedlings growing in soil deficient in that element. Increase of nitrogen supply over the optimum resulted in a decrease in drouth resistance.

7. In the Cloquet nursery, 1-0 seedlings grew best when supplied every week with a fertilizer solution containing 100 p.p.m. of nitrogen. This fertilizer was applied at the rate of 0.45 liters per square foot.

8. Also in the Cloquet nursery, 2-0 seedlings and 1-1 transplants grew best when supplied every week with a fertilizer solution containing 200 p.p.m. of nitrogen, the fertilizer being applied at the rate of 0.45 liters per square foot.

9. The results of the experiments indicate that jack pine stock that has been grown in a soil with a favorable nitrogen supply will have a nitrogen content of 2.25 ± 0.20 per cent. This applies to both one- and two-year-old stock.

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